Current Practice in Medical Science

Vol. 9

Edited by Dr. Darko Nozic

B P International

Current Practice in Medical Science

Vol. 9

Current Practice in Medical Science

Vol. 9

India ■ United Kingdom

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FIRST EDITION 2022

ISBN 978-93-5547-742-2 (Print)
ISBN 978-93-5547-749-1 (eBook)
DOI: 10.9734/bpi/cpms/v9

Contents

PREFACE

This book covers key areas of Medical Science. The contributions by the authors include Exclusive breast-feeding, mother-to-child transmission, infant mortality, Menstrual hygiene, Intratesticular, embryonal rhabdomyosarcoma, Acute myeloid leukemia, myeloid sarcoma , granulocytic sarcoma, Breast cancer, lung cancer, methyl-donors, apoptosis, complementary therapy, Ultrasound, pancreatitis, Meniscal injury, menisectomy, meniscal repair, knee pain, Migraine, neuralgia, headache, Dentistry, health profession, emergency medicine, clinical practice guide, COVID-19, Thyroidectomy, laryngeal nerve injury, vocal cord injuries, Acromegaly, growth hormone, pituitary adenoma, Insulin-like Growth Factor-I, psychiatric disorders, and schizophrenia. This book contains various materials suitable for students, researchers and academicians in the field of Medical Science.

Chapter 1
Print ISBN: 978-93-5547-742-2, eBook ISBN: 978-93-5547-749-1

Exclusive Breast Feeding: Experiences of HIV Infected Mothers in Mangaung, South Africa

Selloane Phakisi [a*] and Johanna M. Mathibe-Neke [b]

DOI: 10.9734/bpi/cpms/v9/2736A

ABSTRACT

The distribution of free formula milk to HIV-positive mothers was subsequently stopped, and these mothers were urged to breastfeed instead. This was also in accordance with the WHO's request that nations implement a single-feeding practise for mothers who are HIV-positive. The objectives of the study were to identify the feasibility of exclusive breast feeding in the context of HIV and the mothers experiences in this regard. Qualitative data was collected through in-depth unstructured interviews at a community health centre among mothers aged 18 years and above, who opted for exclusive breast-feeding. The examination of the data was done thematically. According to the study's findings, mothers reported good feelings like motivation, satisfaction, and knowledge. Some mothers chose not to breastfeed exclusively as a result of unpleasant experiences including anxiety, family pressure, and guilt. Socio-cultural factors and knowledge from healthcare professionals had the biggest impact on the experiences of participating mothers. The study's findings emphasise the necessity of stepping up advocacy, communication, and social mobilisation for exclusive breastfeeding among all communities.

Keywords: Exclusive breast-feeding; experience; mother-to-child transmission; infant mortality.

1. INTRODUCTION

The World Health Organization (WHO) recommends that all mothers, including those who are HIV-positive, breastfeed exclusively [1].

Mother-to-Child Transmission (MTCT) through breastfeeding has been reported to be minimised by exclusive breastfeeding for the first six months, together with anti-retroviral medication for mothers and infant prophylaxis [2]. In spite of this

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milestone, exclusive breast-feeding by HIV-infected mothers still creates a dilemma among health workers and some communities, since MTCT can still occur through breast-feeding [3-5]. Notwithstanding the well-researched benefits of breast-feeding, most notably the prevention of infant mortality and morbidity due to diarrhoea, pneumonia and under nutrition [2], Breast-feeding practices among HIV-positive women are still clouded by fear of HIV infection in some people and communities. The WHO recommends considering the socioeconomic and cultural contexts of the population, the quality of health services, HIV prevalence among pregnant women, and the main cause of child undernutrition and infant and child mortality when deciding whether to replace or breastfeed in HIV-positive women [1,6].

The Prevention of Mother-to-Child Transmission (PMTCT) through breast-feeding should always be balanced with the possible risk of child mortality induced by replacement feeding [1]. Despite its association with MTCT, breastfeeding remains a global challenge [7,8]. Accordingly, the global target for exclusive breast- feeding in the first six months is targeted at a minimum 50% by 2025 and in 2014, it was at 38% [8]. An assortment of challenges regarding exclusive breast-feeding has been identified in Africa and other parts of the world. Cultural beliefs and practices, unsupportive work environments, lack of family support, fear of stigma, lack of knowledge and skills were identified as some of the barriers to breast-feeding in some studies [9-12]. Other factors found to influence the practice of exclusive breast-feeding in low-income countries include employment, education, place of delivery and family pressure [13,14]. Promotion of exclusive breastfeeding is associated with lower risks of diarrhea- and pneumonia-related infant morbidity and mortality than breastfeeding with addition of other fluids and solids in both developed and developing world settings. Exclusive breastfeeding is unlike replacement feeding where reduction of HIV transmission has to be carefully balanced against increased mortality due to other infections. Support for exclusive breastfeeding programs would be strengthened by more rigorous data showing the clear benefits of EBF for HIV prevention [15]. Exclusive breastfeeding promotion efforts among HIV-infected women in sub-Saharan Africa feature additional barriers and challenges. These include stigma associated with any exclusive feeding methods, traditional practices of mixed feeding, divergent messages between counsellors and clinics from whom subjects receive care, and lack of human resources and physical space to deliver sensitive EBF promotion messages [16].

In their meta-analysis on prevalence of key breast-feeding indicators in 29 sub-Saharan African countries between 2010-2015, Issaka et al. [17] found the overall prevalence of breast-feeding to be less than 50%. According to UNICEF, the exclusive breast-feeding rate in South African was at 8% in 2012 [18]. Contrastingly, a study conducted by Siziba et al. found the exclusive breast-feeding rate to be 12% in 2013 in four of the nine South African provinces [19].

Additionally, Southern and Eastern Africa are the most affected by the HIV burden, and also among the highest in child mortality [6]. Therefore, it is imperative that HIV prevention is balanced with other child survival strategies

such as breast- feeding. Victora et al. [7], assert that only 37% of children under six months are exclusively breast- fed in low-income and middle-income countries. In their study on constraints to exclusive breast- feeding in Nigeria, Agunbiade et al. [20] found that only 19% of breast-feeding mothers whose babies were below six months practised exclusive breastfeeding. Prior to 2012 in South Africa, HIV- infected mothers who opted for replacement feeding received free commercial formula from public health facilities for six months [21]. Through the Tshwane Declaration of August 2011, the South African government committed to promote, support and protect breast-feeding20. Subsequently, the provision of free formula to HIV-infected mothers who choose formula feeding was discontinued, and all mothers were encouraged to breast-feed [22]. The Declaration was also in response to the WHO (2010) recommendation that national authorities should implement a single feeding practice for HIV-infected women1. This was a new approach to mothers and some health professionals. The researchers in this study also observed that some HIV-infected mothers were still despondent about breast-feeding. Therefore, the practice needed to be explored and explained further, so that it is understood better by its intended beneficiaries. In this regard, the experiences of the mothers would provide an understanding of some of the barriers to exclusive breast-feeding, such that best practices could be enhanced. These empirically generated experiences would in turn inform the policymakers concerning the salient factors contributing to non-adherence to exclusive breast-feeding by HIV infected mothers. These factors could be inimical to overall child survival.

2. METHODS

2.1 Study Design

A qualitative study was conducted to describe and explore the exclusive breast-feeding experiences of HIV- infected mothers during the first six months of the infants‘ life. Purposive sampling was applied in the participants‘selection process, based on the researcher‘s predetermined selection criteria.

2.2 Study Settings and Population

The study was conducted in an urban community health centre in Mangaung, South Africa. HIV- infected mothers aged 18 years and above who chose to exclusively breast-feed their babies in their first six months, were included to participate voluntarily in the study. *Ergo,* their infants had to be older than six months at the time of data collection.

2.3 Data Collection

Individual unstructured in-depth interviews were conducted in a private consulting room of the community health centre in 2014. Demographic data was first obtained from each participant, stating age, marital status, education level and number of children. A grand tour question was: Kindly describe your exclusive breastfeeding experiences in the first six months after your baby‘s

birth?. Probing questions were asked until saturation point after interviewing fifteen HIV- infected mothers. The audio-recorded interviews were conducted in South Sotho (the local language), translated to English, and transcribed verbatim.

2.4 Data Analysis

Each interview transcript was systematically coded, and concepts and connections identified, followed by generation of themes [23,24].

3. RESULTS

Table 1 presents the socio demographic characteristics of the participants, of whom 11 were between the ages of 20-30, and 4 (four) were aged 31-40 years. Six (6) were married, and 9 (nine) were single. All participating mothers had formal education, 1 (one) primary, 13 high school, and 1 (one) tertiary. Unemployment was high, with 13 mothers unemployed. Additionally, all mothers had attended antenatal clinic, 6 (six) of whom already knew their HIV-positive status on the first visit while 9 (nine) were diagnosed positive during pregnancy. Three themes emerged from the collected data. These were positive experiences, negative experiences, and challenges encountered.

3.1 Positive Experiences

3.1.1 Well informed

Mothers reported that they were well informed about exclusive breast-feeding and were aware of the benefits thereof, including PMTCT interventions. They mostly concurred both the information and interventions had a positive impact on their breast-feeding experiences. One of the mothers stated:

I was taught at the clinic that breast milk was good, and that the Nevirapine syrup that the baby was getting would protect my baby from getting HIV. I was also told not to give the baby anything except the breast-milk for six months.

3.1.2 Satisfaction and motivation

Satisfaction and motivation were the result of being well informed, as mothers were not anxious about their babies contracting HIV. Disclosure of HIV status also had a major impact on the satisfaction and motivation because mothers who disclosed their status received support, and were not always worried about people‘s reaction concerning their HIV status. Collectively, these positive experiences also contributed to mothers‘ adherence to breast-feeding as indicated below:

I wanted to give my baby the best I could, so I was very content and happy about breastfeeding.

One mother who was misinformed about the duration of breast-feeding her baby and had to discontinue abruptly at six months, expressed guilt about not being able to continue breast- feeding beyond six months.

It's so painful to me that I had to deprive my baby of love because of the HIV. It made my life difficult in many aspects ... I had already accepted my status, which was yet another setback. I felt sad and I disclosed my status to everyone. I talk a lot about it, even to strangers. Some people used to comment about my breastfeeding while I was HIV-positive. I would tell them that my child was safe because I was taking ARV's and not mixed feeding.

Table 1. Socio-demographic characteristics of hiv- infected mothers in mangaung, free state province, South Africa (N=15)

Criterion	Characteristic	*Frequency*
Age	20-30 years	11
	31-40 years	4
Marital status	Married	6
	Single	9
Level of education	Primary School	1
	High school	13
	Tertiary	1
Employment status	Employed	2
	Unemployed	13
Period of diagnosis of HIV infection	Known positive on first antenatal visit	6
		9
	Diagnosed HIV in pregnancy	3
Planned versus unplanned pregnancy	Planned	12
	Unplanned	

3.2 Negative Experiences

3.2.1 Anxiety

Some of the HIV-infected mothers experienced anxiety despite the health education they received. They were still fearful of their babies contracting HIV. Non-disclosure of HIV status also contributed to perceived lack of support and negative experiences of stigma and discrimination should their HIV-positive status be known.

I was anxious because I knew there is a possibility of my baby's infection from breast milk .... My worst time was when I had to fetch the results of the baby's first test. I was very anxious and did not sleep that night. even guilty when I had to stop breast- feeding. It was like the first time the status was revealed to me.

3.2.2 Feeding incongruence

A mother who gave birth to preterm twins, and was well informed about exclusive breast-feeding, discovered that her babies were given formula at the hospital while she was also providing expressed breast milk to them.

On several occasions when I took breast milk to the nursery, I found that my babies had been fed with formula. When I asked about it, the nurses told me that my milk has never been sufficient for those two babies … I was told several times at the clinic that it should either be breast milk or formula alone, that breast milk was best, and never to mix feed because babies can contract HIV and other infections … Why was I not told from the beginning that my milk was not enough?

.... Why was I advised on something that was not feasible?

3.2.3 Family pressure and conflict

Other practices associated with exclusive breast- feeding resulted in family pressures and conflict, leading to cessation of breastfeeding before six months. Such practices included babies not getting water, expressing breast milk, and mothers going back to work.
My father was very dissatisfied about the issue of expressing breast milk. He said it was disgusting to wash the utensils with breast milk where we wash other dishes and keeping the milk in the fridge with other food items.

A mother who had to go back to work left the baby with her mother who was also against expressing breast milk. She had to stop breast- feeding before six months.
There are some things that made me suspect that the baby did not even get that expressed breast milk, but I kept quiet as there was nothing I could do. I then stopped breast-feeding at four months.

3.3 Challenges Encountered

3.3.1 Mixed feeding

Mixed feeding resulted from factors such as family pressure and the perception that babies were not provided with enough milk. One mother related her mother‘s insistence that the baby could not survive without water, an insistence to which she eventually surrendered.

I experienced some problems when my mother came to visit. She fiercely contended that a child could not survive without water … My mother ended up giving the baby some water and I had to stop breastfeeding.

Mothers also related how they had to introduce formula milk. They believed their babies cried a lot because they received insufficient milk.

I started giving my baby formula at one month because he would not stop crying. So I believed he was not getting enough milk.

3.3.2 Misinformation

Some of the mothers indicated that they only breastfed for six months. They were taught at the clinic that HIV-infected mothers were only to breast-feed for six months, which led to unhappiness. This is contradictory to WHO and South African guidelines, which state that HIV- infected mothers can breast-feed exclusively for six months, introduce complementary feeding, and continue breast-feeding for about two years [6,25]. One mother stated:

Breastfeeding was a good experience for me. I became very sad when I had to discontinue the practice. I wanted to continue, but was told never to exceed six months. So, it was difficult for me and my baby, who would cry for hours and … I even wished I had not started at all … I got this information from the clinic

3.3.3 Resuming work

Exclusive breast-feeding impacted negatively on working mothers when they had to return to work. They were informed about expressing breast milk and leaving it with the caretakers to feed the babies during the day. They were compelled to stop breastfeeding due to family pressures and mistrust. One of the mothers averred:

I returned to work and left my baby with my mother, who insisted that the baby needs water from birth and some porridge from four months. I realised I could be endangering my child because it was obvious my mother was going to give her some other things, so I decided to stop breast-feeding.

4. DISCUSSION

Participating mothers had a variety of experiences influenced by a number of factors. Data from this study shows some socio-demographic characteristics did not impact HIV-infected mothers‘ exclusive breast-feeding experiences in the first six months of the infant‘s life. These characteristics ranged from level of education to whether or not the pregnancy was planned.

Participants aged thirty to forty years had more positive experiences. Married mothers experienced less challenges. In this regard, being married or staying with a partner was a predictor of more positive experiences, adhering to exclusive breast-feeding and more support on the chosen method of feeding; this was consistent with some findings from other studies [26,27]. Some of the fifteen participating mothers were employed. Their negative experiences started when they had to return to work, and both had to discontinue breast- feeding before six months. Contrastingly, the mothers who had known their status before pregnancy had fewer negative experiences and challenges.

Furthermore, positive experiences resulted from the health education provided regarding exclusive breastfeeding, disclosure of HIV status, and the level of support provided. The mothers were eager to breast-feed exclusively and less

anxious about HIV transmission to their babies because of the capacity of antiretroviral therapy and exclusive breastfeeding to minimise the risk of MTCT. Disclosure of HIV status also led to more positive experiences, such as support, which resulted in adherence to exclusive breast-feeding. A study conducted in South Africa by Sibeko et al. [3] revealed that non-disclosure of HIV status was also found to be an obstacle to PMTCT. Incorrect information provided to mothers by some healthcare workers, that HIV-positive mothers should only breast-feed for only six months, contributed to negative experiences; such as abrupt weaning which deprived the babies of the continued benefits of breast milk. Correct information should always be provided to communities, because misinformation could have detrimental effects on the health outcomes of the very communities. In addition, healthcare workers' attitudes, skills and knowledge have an impact on the sustenance of exclusive breastfeeding [7]. This was attested to by the experiences and the remarks of the mother whose babies were mix-fed at the hospital, with the mother concluding that she was taught and expected to practice something that was not feasible.

Family pressure, which emanated from previous culturally induced practices, also had negative implications, which led to strained relationships, mixed feeding and early cessation of breast-feeding. This demonstrates there was still some ignorance concerning exclusive breast- feeding in general, and not only among HIV- infected mothers. These pressures also impacted on mothers who had to return to work and leave the babies with caretakers. The totality of the participating mothers' experiences highlights the issue of extending maternity leave to six months. A breast-feeding review of six countries by Mangasaryan et al. [10] also revealed ignorance by some healthcare providers, lack of family support, work and limited maternity leave as obstacles to breastfeeding.

5. CONCLUSION

The study demonstrated that although the mothers were receptive to the practice of exclusive breastfeeding, there were some factors which still affected its sustenance. Provision of health education and counselling contributed positively to the mothers' experiences. However, the fact that some of the education was incorrect, had negative effects. Although the study was mainly on HIV- infected mothers, the data shows that health workers have to continually be informed on infant and young child feeding policy. Lack of knowledge by other family members also impacts negatively on exclusive breastfeeding, and highlights the need to intensify community mobilisation and participation. Some mothers were still anxious about MTCT, which highlights the need for continuous counselling throughout pregnancy and breast-feeding. The study results also demonstrate the importance of considering the socio-cultural contexts of health recipients when formulating policies. Working mothers have to be supported at policy-making level by considering extension of maternity leave to at least six months. Provision of longer lunch breaks for breast-feeding mother has been implement by some employers, but some mothers work far from home and cannot benefit from such policies which benefits some.

6. LIMITATIONS

The study was conducted in one community health centre with HIV-infected mothers aged 18 years and above. The participating mothers were mainly from selected townships and informal settlements around Mangaung, which could affect the generalisability of the findings to all mothers in the surrounding areas. The inclusion of mothers younger than eighteen years could have provided more value as this an age cohort most likely to be victims of unplanned teenage pregnancies. This study also focused on HIV-positive mothers who opted for exclusive breast-feeding, and not those who had opted for exclusive formula feeding. Therefore, the findings cannot conclusively determine exclusive breastfeeding for the first six months of the infant's life to be generally accepted by mothers.

ETHICAL CONSIDERATIONS

Approval to conduct the study was obtained from the Head of the Free State Department of Health, while ethical clearance was granted by the University of South Africa, with clearance number: HSHDC/270/2013. Participation was voluntary and informed consent was sought from each participant. Confidentiality, privacy and anonymity were ensured by conducting the interview privately and the use of pseudonyms in the audio recordings.

ACKNOWLEDGEMENTS

The authors acknowledge the Free State Department of Health and all relevant authorities for granting permission for this study, as well as the mothers who willingly participated.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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DISCLAIMER
This chapter is an extended version of the article published by the same author(s) in the following journal.
African Journal of Reproductive Health, 23(4): 27, 2019.

Chapter 2
Print ISBN: 978-93-5547-742-2, eBook ISBN: 978-93-5547-749-1

Menstrual Hygiene among Reproductive Age Group Women in a Rural Area, Tamil Nadu

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DOI: 10.9734/bpi/cpms/v9/7271F

ABSTRACT

The purpose of this study is to evaluate menstrual hygiene practices and the impact of socio-demographic factors on menstrual hygiene among women of reproductive age group in a rural area. Menstrual hygiene management should be an imperative part of healthcare to safe guard the reproductive health of women. It is a cross-sectional community-based study that was carried out among 200 women of reproductive age group (15-45 years) in a rural field practice area of VMKV Medical College Hospital, Salem, using a simple random sampling technique. Majority of the study population, 36% (72/200) belonged to the 21-30 years age group. About 75% (150/200) were married. Majority of women 35% (70/200) were unskilled workers, 43.3% (86/200) had primary education and 54.3% (108/200) belonged to lower middle class. The mean age of menarche among the reproductive age- group women was 13.15 years. Majority of women 51.8% (104/200) used cloth during menstruation, about 45.7% (91/200) used the same cloth by washing and reusing every month. Socio-demographic characteristics and hygiene behaviours had a substantial impact on women during menstruation ($P < 0.001$). It was observed that the majority of rural women were engaged in unhygienic behaviour during menstruation. Hence, efforts should be made to create awareness about using sanitary pads and follow hygienic practices during menstruation,as a part of menstrual hygiene management (MHM) ,along with improving female literacy and general living conditions.

Keywords: Menstrual hygiene; reproductive age-group women; rural area.

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1. INTRODUCTION

Menstrual hygiene is alarmingly lacking among rural populations, so policy-making and awareness-raising initiatives must start right away [1].

Menstrual hygiene is a taboo subject; many South Asian women feel awkward talking about it in public. Naturally, things that are not discussed in public are more likely to be ignored or given little importance. This is compounded by gender inequality, which excludes women and girls from decision-making processes. Hygiene-related practices of women during menstruation are of considerable importance, as it has a health impact in terms of increased vulnerability to reproductive tract infections (RTI). The interplay of socio-economic status, menstrual hygiene practices, and RTI are noticeable. Today millions of women are sufferers of RTI and its complications and often the infection is transmitted to the offspring of the pregnant mother [2].

Reproductive Tract Infections (RTIs), which have become a silent epidemic that devastate women's life, are closely interrelated to poor menstrual hygiene. The use of rags and old clothes is a rule rather than exception in rural areas of India. Unclean rags and old clothes increase the chances of RTIs including urinary, vaginal, and perineal infection. Very often, serious infections are left untreated and may sometimes lead to potentially fatal toxic shock syndrome. Untreated RTIs are responsible for 10-15% of fetal wastage and 30-50% of prenatal infection. Increasingly, RTIs are also linked with the incidence of cervical cancer, HIV/AIDS, infertility, ectopic pregnancy, and a myriad of other symptoms [3].

Women having better knowledge regarding menstrual hygiene and safe practices are less vulnerable to RTI and its consequences. Therefore, increased knowledge about menstruation right from childhood may escalate safe practices and may help in mitigating the sufferings of millions of women [3].

Menstrual hygiene depends on the type of material used, frequency of changing the material, bathing daily, washing the genital area and proper disposal of used materials [4]. The first step is raising awareness, hygiene education and promotion, the provision of affordable and accessible products and facilities, and waste management.

There is a cyclical causal relationship between the neglect of menstrual hygiene and low levels of awareness amongst communities, practitioners, and policymakers, which needs to be broken. The negative effects of this neglect are far-ranging on the lives of women and on the achievement of wider development goals [5].

Menstrual hygiene management should be an imperative part of healthcare to safe guard the reproductive health of women. Medical and public health research supports an ongoing need for health promotion in meeting menstrual hygiene needs, including menstrual hygiene management (MHM) education and the adoption of reusable sanitary napkins [6,7].

This study aims to assess the hygienic practices during menstruation and the influence of socio-demographic factors,so that menstrual hygiene needs are understood and proper menstrual hygiene management education(MHM) is given to the rural women.

2. SUBJECTS AND METHODS

This is a cross-sectional, community-based study conducted in Oct-Dec 2013 for a period of 3 months among 200 women of reproductive age group (15-45 years) in a rural field practice area, VMKV Medical college, Salem after obtaining ethical clearance from the institution and informed consent from the reproductive age-group women. The sample size 200 was calculated by taking into consideration 19% of women under 15-45 years in the community at 95% Confidence Interval and 3% permissible error covering ± 1.96 under normal distribution curve, with the application of the formula [$\pm 1.96 \sqrt{Pq/n} = \pm 0.03$].

A pretested structured questionnaire was used and validated by standard questions on menstrual hygiene along with testing by pilot study. By using simple random sampling technique, women were selected and interviewed regarding their socio-demographic history, age of onset of menstruation, and menstrual hygiene-related practices such as use of cloth or sanitary pads during menstruation, disposal of pads, reuse of cloths, daily bathing using soap, and genital hygiene by washing with soap and water during menstruation.

3. ANALYSIS

3.1 Statistical Analysis

Statistical tests like Chi-square and Proportions were used for analysis by using SPSS software.

4. RESULTS

Table 1 shows the socio-demographic profile of the study population. It was found that majority of study population 36% (72/200) belonged to 21-30 years of age. This was followed by women of age 31-40 years, 34.5% (69/200). Young girls of 15-20 years constituted 22% (44/200) and women of age group 41-50 years were of 17.5% (15/200). The mean age of reproductive age-group women was 29.2 years.

Majority of women 60% (120/200) attained menarche at the age of 13-15 years, followed by 37% (74/200) at the age of 10-12 years and 3% (6/200) at the age of 16 and above. The mean age of menarche among reproductive age group women was 13.15 yrs.

About 75% (150/200) were married, while 25% (50/200) were unmarried. Majority of women had primary education 43.3% (86/200), followed by 28.5% (57/200) higher secondary schooling, 20.5% (41/200) illiterate women, and 8% (16/200)

graduates. The number of unskilled workers were 35% (70/200), followed by 28.5% (57/200) semiskilled workers, 22.5% (45/200) skilled workers, and 14% (28/200) students.

It was found that majority of women 30% (60/200) belonged to Lower Middle class, followed by 24.5% (49/200) belonged to Lower Upper class and Upper class. About 14% (28/200) belonged to Lower lower class and 7% (14/200) belonged to Upper Middle class.

Table 1. Socio- demographic profile of the study population

Socio–demographic profile	**Number of women, *n*=200**	**Percentage**
Age group		
15- 20	44	22.0
21- 30	72	36.0
31- 40	69	34.5
41- 50	15	7.5
Age of menarche		
10- 12	74	37
13- 15	120	60
16 and above	6	3
Marital status		
Married	150	75
Unmarried	50	25
Educational status of women		
Illiterate	41	20.5
Primary	86	43.0
High school	57	28.5
Graduate	16	8.0
Women occupation		
Unskilled	70	35
Semiskilled	57	28.5
Skilled	45	22.5
student	28	14
Socioeconomic status of women		
Lower lower	28	14
Lower Upper	49	24.5
Lower middle	60	30
Upper middle	14	7
Upper class	49	24.5

Table 2 shows menstrual hygiene practices among study population. Majority of women 52% (104/200) used cloth during menstruation. About 35% (70/200) used sanitary pads, while 13% (26/200) preferred both cloth and sanitary pads. The number of women who used same cloth every month after washing and reusing constituted 45.5%(91/200), while 19.5% (39/200) used new cloth each time of menstruation. The number of women using antiseptic lotion to wash the cloth

were 13.4% (27/200) only, while 51.5% (103/200) did not use any antiseptic lotion to wash the cloth used during menstruation.

Majority of women 32% (64/200) changed 2-3 sanitary pads per day, followed by 13.5% (27/200) women who changed less than 2 sanitary pads per day, and 2.5% (5/200) women changed 4-5 sanitary pads per day. It was found that among women who used sanitary pads majority of them 32.5% (65/200) threw it in dust bin and 15.5% (31/200) burned it. Among those who used clothes, 45.5% (91/200) women washed and reused it ,while 19.5% (39/200) used new cloth. It was found that around 16.5% (33/200) had poor genital hygiene during menstruation, while 83.5% (167/200) maintained genital hygiene during menstruation by washing after changing of the pads.

Table 3 shows influence of socio-demographic factors and hygienic practices during menstruation. It was found that among 70 unskilled workers, majority 64.3% (45/70) used cloth during menstruation, while among 57 semiskilled workers, majority 54.4% (31/57) used cloth, whereas among skilled workers and students, majority 53.3% (24/45) and 71.4% (20/28) used sanitary pads during menstruation, respectively. This difference was found to be statistically significant ($P < 0.001$).

It was found that among illiterate women, majority 95.1% (39/41) used cloth during menstruation while among those who had primary education, majority 59.3% (51/86) used cloth whereas among those who had completed high school and graduation, majority 70.2% (40/57) and 93.7% (15/16) used sanitary pads during menstruation, respectively. This difference was found to be statistically significant ($P < 0.001$).

It was found that among those who were married, majority 62.7% (94/150) used cloth during menstruation, while among those who were unmarried, majority 60% (30/50) used sanitary pads during menstruation. This difference was found to be statistically significant ($P < 0.001$).

It was found that socio-economic classes influenced on menstrual hygiene practices. Among Lower lower, Lower Upper, and lower middle class women, majority 100% (28/28), 53% (26/49), and 55% (33/60) women used cloth during menstruation, while among those of upper middle class and upper class, majority 85.7% (12/14) and 65.3% (32/49) used sanitary pads during menstruation. This difference was found to be statistically significant ($P < 0.001$).

5. DISCUSSION

Menstrual hygiene and management is an important issue that is insufficiently acknowledged and has not received adequate attention [8]. Hence, this study was conducted,where majority of women 120 (60%) attained menarche at the age of 13-15 years, followed by 74 (37%) at the age of 10-12 years and 6 (3%) at the age of 16 and above. The mean age of menarche among reproductive age group women was 13.15 years.

Table 2. Practices of menstrual hygiene among study population

Menstrual hygiene practices	Number of women, *n*=200	Percentage
During menstruation		
Use cloth	104	52
Use sanitary pad	70	35
Use both cloth and sanitary pad	26	13
Use same cloth every month		
Yes	91	45.5
No	39	19.5
Use antiseptic lotion for cloth		
Yes	27	13.4
No	103	51.5
Number of pads changed every day		
<2	27	13.5
2- 3	64	32.0
4- 5	5	2.5
Disposal practices		
Throw the pad or cloth	65	32.5
Burn it	31	15.5
Wash and reuse the cloth	46	23.0
Use new cloth each time	84	42.0
Genital hygiene		
Yes	167	83.5
No	33	16.5

Similarly, a study by Kamaljit et al. found that the age of menstruating girls ranged from 10 to 15 years with maximum number of girls falling between 12 and 15 years of age, and the mean age of menarche of the respondents has been observed as 12.5 years [9]. A similar study conducted by Deo et al. [10]. reported that the age of menstruating girls ranged from 12 to 17 years, with maximum number of girls between 13 to 15 years of age, whereas in a study carried out in Rajasthan by Khanna et al., [11] the mean age at menarche was found to be 13.2 years.

Our study shows that majority of the women preferred cloth rather than sanitary pads as menstrual absorbent. Only 35% women used sanitary pads during menstruation. It was observed that the usual practice was to wash the cloth with soap after use and keep it at some secret place till the next menstrual period. To keep the cloth away from prying eyes, these are sometimes hidden in unhygienic places. Privacy for washing, changing, or cleaning purpose is something very important for proper menstrual hygiene. In a study conducted in Rajasthan by Khanna et al., [11] three-fourths of the girls used old cloth during their periods and only one-fifth reported using readymade sanitary pads. Similarly, a study regarding menstrual hygiene practices by Kamaljit et al. [9] found that 68.7% girls

used sanitary pads and 30 (10.0%) respondents practicing any cloth or rag/cotton during menstruation.

Regarding the method of disposal of the used material, most of the women 45% reused cloth pieces. In a similar study conducted among 664 schoolgirls aged 14-18 years in Mansoura, Egypt by El-Gilany et al., [12] the different aspects of personal hygiene were generally found to be poor, such as not changing pads regularly or at night and not bathing during menstruation, with lack of privacy being an important problem.

A study by Ray Sudeshna et al. found good menstrual hygiene was more among girls with literate mothers, girls studying in more than grade 10 in school, having prior knowledge about menstruation before menarche, usage of proper sanitary latrine at home, and exposure to advertisements promoting usage of sanitary towels in mass media [13].

A study by Shamima Yasmin et al. found that out of 147 respondents, 62 (42%) girls were aware about menstruation prior to attainment of menarche. Hand-washing was regular among 91.8% but 16.3% washed only with water. Similarly, washing of private parts were regular among 76.9% but 74.1% used only water no soap, there is significant relationship between hygienic practices followed and presence of continuous supply of water and presence of exclusive toilet of their family [14].

A study by Salve et al. found that 93 (49%) rural and 94 (71%) urban girls had started menarche and regularities of menstruation was better in rural girls, i.e. 87 (94%) compared to urban girls, 53 (56%). Percentage of using market available sanitary napkins was more in urban girls 56 (60%) compared to rural girls 6 (06%), whereas homemade sanitary napkins were used by 87 (94%) rural girls and 38 (40%) urban girls and this difference was statistically significant amongst rural girls. Female teacher was the main source of knowledge 89 (47%) in rural areas while it was the mother in urban area 48 (36%). Knowledge about reproductive system, determination of fetal sex, age of marriage, etc., was better amongst urban girls. Social taboos such as separate sitting, restriction on attending school, and social functions were more amongst rural girls while sanitary facilities such as attached toilet, full wall bathroom, sufficient water, etc., were less in rural areas [15].

Out of total 360 adolescent girls, 257 (71.39%) girls have attained menarche. Maximum number of girls (72.77%) attained menarche in the age range 12-14 years. About 15.96% girls reported blood flow for more than 5 days. In 66.54% girls, menstrual cycle was of 28-32 days [15].

Table 3. Relationship of socio-demographic profile and practice of menstrual hygiene of the study population

Sociodemographic variables	Menstrual hygiene practices (%)			Total	Level of
	Use cloth	Use pad	Use both cloth and sanitary pad	*n*=200	significance
Women occupation					
Unskilled	45 (64.3)	15 (21.4)	10 (14.3)	70	x^2=32.85
Semiskilled	31 (54.4)	11 (19.3)	15 (26.3)	57	*P*<0.001
Skilled	20 (44.4)	24 (53.3)	1 (2.3)	45	
student	8 (28.6)	20 (71.4)	0 (0)	28	
Total	104	70	26	200	
Women education					
Illiterate	39 (95.1)	2 (4.9)	0 (0)	41	x^2=27.18
Primary	51 (59.3)	13 (15.1)	22 (25.6)	86	*P*<0.001
High school	13 (22.8)	40 (70.2)	4 (7.0)	57	
Graduate	1 (6.3)	15 (93.7)	0 (0)	16	
Total	104	70	26	200	
Marital status					
Married	94 (62.7)	40 (26.7)	16 (10.7)	150	x^2=16.34
Unmarried	10 (20)	30 (60)	10 (20)	50	*P*<0.001
Total	104	70	26	200	
Socioeconomic status					
Lower lower	28 (100)	0 (0)	(0)	28	x^2=34.56
Lower Upper	26 (53.0)	14 (28.6)	9 (18.4)	49	*P*<0.001
Lower middle	33 (55)	12 (20)	15 (25)	60	
Upper middle	2 (14.3)	12 (85.7)	0 (0)	14	
Upper class	15 (30.6)	32 (65.3)	2 (4.1)	49	
Total	104	70	26	200	

A study by Keerti Jogdand et al. found that only 36.19% girls were aware regarding menstruation prior to the attainment of menarche. About 53.7% girls reported use of sanitary pads during menstruation, 34.63% girls reported use of old clothes during menstruation, and 11.6% reported of having used both, similar to youngsters in our study. About 78.99% girls were not allowed to attend religious occasions. 22.97% and 20.63% girls were restricted from doing routine household work and playing, respectively. [16].

6. CONCLUSION

Unhygienic menstrual practices, a very important risk factor for reproductive tract infections, is a vital aspect of health education for adolescent girls. Educational television programs, trained school nurses/health personnel, motivated school teachers, and knowledgeable parents can play a very important role in transmitting the vital message of correct menstrual hygiene to the adolescent girls of today.

Efforts such as improving the female literacy and health education on the various risk factors should be made by the policy makers to increase the menstrual hygiene among rural population. Adoption of high quality menstrual hygiene will play an important role in prevention of RTI and Cancer of cervix among the women population. Therefore, promoting positive attitudes towards management of menstruation and related problems among the reproductive age group women is the need of the hour.

7. RECOMMENDATIONS

A separate National health policy concentrating on improvement of menstrual hygiene, thereby prevention of reproductive tract infections, is needed along with continued health education, to measure the success of interventions aimed at improving the menstrual hygiene practices among women. Establishment of a comprehensive school health education program with instruction in hygienic practices related to menstruation is the need of today.

Universalized use of sanitary pads or absorbent material needs to be advocated to every women by making the easy availability through social marketing,as a part of Menstrual hygiene management.

ACKNOWLEDGEMENT

The authors are grateful to the Head of the institution and Head of Community Medicine Department of VMKV medical college, Salem, Tamil Nadu,during the study period, for their permission and encouragement to conduct the study and they also thank all the women for their co-operation and support.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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She is currently working as Professor and Head in the Department of Community Medicine at VMKVMCH, Salem, Tamil Nadu, India. She was an Assistant professor in Vinayaka Mission's Medical College, Salem and Associate Professor in Annapoorna medical college, Salem, India. She was selected as Professor and HOD of Community Medicine Department at ESIC Medical College, Chennai and she worked for a period of 1year in Chennai. She completed MBBS from Bangalore Medical College, Bangalore, Karnataka, India in 2001,with gold medal in ENT and Master degree(MD) in Community Medicine from Karnataka Institute of Medical Sciences , Hubli, Karnataka, India in 2006,with state 2nd rank in MD exams. She is interested in Reproductive & Child Health Programme, Artificial Intelligence, Communicable diseases, Non Communicable diseases and Nutrition. She attended as an invited speaker, to share her research experience on ''Community based study on Reproductive Tract Infections among women'' in an International Conference of Gynaecology and Obstetrics (ICGO-2013),under 1st Annual Global Health Conference, held at Dalian, China from Oct 11th-Oct 15th 2013. She has won best paper presentation award as PG in KACH (Karnataka Association of Community Health) conference and in Indian Association of Public Health conference, Cochin, Kerala, India as a faculty. She has been also awarded Dr APJ Abdul Kalam award for teaching excellence in 2017 by Marina Labs Research and Development, "Dr. Sonaji Jogadand Prize" award in the category of "Occupation/Environmental Health" for her oral presentation in 22nd Annual Maharashtra State Joint Conference of IAPSM & IPHA – 2021 and Best Researcher award 2021 by VMKVMCH, Salem.
She has the credit of conducting an Integrated National Public Health Conference, first time in India, as an Organizing Secretary at VMKV Medical College, Salem, Tamil Nadu on Dec 2nd and 3rd 2017. She is an Editor in Chief for University VMRF (DU) News Letter – VINSAGA. She has 22 published papers in National indexed journals and 10 papers in International indexed journals.

DISCLAIMER
This chapter is an extended version of the article published by the same author(s) in the following journal.
Journal of Basic and Clinical Reproductive Sciences. 3(2), 2014.

Chapter 3
Print ISBN: 978-93-5547-742-2, eBook ISBN: 978-93-5547-749-1

Testicular Tumor in Children: A Rare Case Report

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DOI: 10.9734/bpi/cpms/v9/7332F

ABSTRACT

Male genital tract mesenchymal neoplasms are uncommon, with the majority occurring in the paratesticular and testicular adnexa. The most common sarcomas in children with this location are paratesticular embryonal rhabdomyosarcoma (RMS). Without any personal pathological history, a 4-year-old boy reported pain and a 2 month-old rise in the size of his left testicle. Physical examination revealed that the left scrotal sac was swollen, uncolored, indurated, and not particularly uncomfortable. No changes were visible in the right testicle.
The RMS is the second most common soft tissue tumour in children, after the head and neck region, and it most frequently develops in the genitourinary system. It is rare to develop primary intratesticular rhabdomyosarcoma.

Keywords: Intratesticular; embryonal rhabdomyosarcoma.

1. INTRODUCTION

Testicular tumours are classified into five types: germ cell tumours (90 percent), stromal tumours of the sex cord, mixed tumours of the sex cord stroma and germ cells, primary tumours not specific to the testicle, and metastatic tumours [1]. Mesenchymal neoplasms of the male genital tract are rare, and mostly located in structures for testicular and testicular adnexa, within the sarcomas; the most frequent in the child with this location is the paratesticular embryonal rhabdomyosarcoma (RMS) [1].

Rhabdomyosarcomas are the most common soft tissue sarcomas in children under 15 years. It is estimated that they represent approximately 8% of malignant childhood tumors, with an incidence of 4.5 cases per million children and

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adolescents [1,2]. Rhabdomyosarcomas constitute a unique group of soft tissue neoplasms that share a propensity to undergo myogenesis, a well-defined biologic process that primarily occurs during embryonal and fetal development. As a result, these neoplasms tend to resemble stages of muscle formation more akin to prenatal than postnatal life [3-5]. The RMS are more frequent in the head and neck, followed by the genitourinary region [6]. Within the genitourinary region, the ones located in the bladder and paratesticular are the most frequent [7]. Treatment is intense, with a nevertheless poor prognosis on high-risk patients. Discovery of new therapies would benefit from additional preclinical models.

The intratesticular RMS is uncommon and is barely reported in the literature [7-12]. The origin of a primary intratesticular sarcoma is derived from two fundamental theories, by malignant transformation of a germinal neoplasm of the teratoma type or due to a process of dedifferentiation of multipotential germ cells [8].

In a study conducted by the cancer registries of the Cancer Fighting Society (centers Quito, Cuenca, Loja, and Manabí), showed that the most frequent age of childhood tumors was 1-4 years old, being the most frequent neoplasms, leukemias, lymphomas, and the central nervous system. The sarcomas were in 9th place with 5.1% of the total registered cases [12].

Intratesticular sarcomas that are not associated with germ cell tumors or retroperitoneal metastases are a unique type of intrascrotal sarcoma 3. Prince in 1942 reported that 1 to 2% of testicular tumors are sarcomatous, and only a small percentage of these are rhabdomyosarcomas, with an age of presentation that varies from 21 months to 67 years old [9,10].

2. CASE REPORT

A 4-year-old male with no significant history, complained of pain and increased left testicular volume of two months evolution. Upon physical examination, the left scrotal sac was enlarged, without change in coloration, indurated, not very painful. The right testicle showed no alterations. The laboratory tests were found within normal range, except for the lactate dehydrogenase (LDH) that was high (210UI/L).

The testicular ultrasound showed an interface line at the level of the left testicle, with a well-defined round heterogeneous mass with hyper echoic points inside, with increased vascularization compared to the rare residual testicular parenchyma. The abdominopelvic computed tomography showed a marked increase of the left testicle, almost completely replaced by tumor mass (Fig. 1).

A left radical orchiectomy was performed. The surgical piece measured 6.0 x 2.3 x 1.5 cm. The gross appearance showed that the testicle was replaced by an oval formation of 5 x 2 x 1.5 cm; yellowish white color with cystic degeneration in the upper pole, which covered more than 95% of the testicular parenchyma, no necrosis was identified. The spermatic cord was free of lesion.

In the histological study, we have found a poorly differentiated, hyper cellular neoplasm with medium, small round blue highly mitotic cells. The tumor is formed almost exclusively by primitive cells without rhabdomyoblastic differentiation (Figs. 2 and 3).

The immunohistochemistry showed positivity for Vimentin and Desmin being negative for the rest of the markers, including the specific muscle alpha-actin (Figs. 4 and 5).

Diagnosed as an embryonic rhabdomyosarcoma of small cells with alveolar pattern, intratesticular with infiltration of epididymis, invasion of tunica albuginea, and tunica vaginalis, with several foci of vascular invasion. No invasion to the testicular hilum, without infiltration to the spermatic duct. No per neural invasion. The margins of the tunica vaginalis were compromised.

The patient after radical surgery, once the diagnosis was confirmed, and the extension study was completed; the adjuvant treatment was performed following the current SIOP protocol in our center at the time of diagnosis. It is currently in the 7th VAC cycle (Vincristine, Actinomycin D and Cyclophosphamide) with complete remission of the disease and no metastases.

Histologically, according to the Intergroup Rhabdomyosarcoma Study Group (IRSG) has subdivided rhabdomyosarcoma into low, intermediate, and high-risk groups for the purpose of protocol placement in pediatric patients. [6,13].

Based on clinicopathologic variables including histologic type, extent of surgical control of local disease and primary tumor site. Botryoid variant has a particularly good prognosis, although may have late relapse. Other favorable prognostic factors are age (between 1 and 9 years), orbital and paratesticular location and absence of metastatic disease at the time of resection.

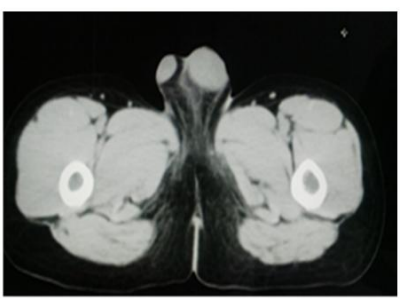

Fig. 1. Abdominal-pelviccomputed tomography, where is observed the nearly total replacement of the testicular parenchyma by the tumor

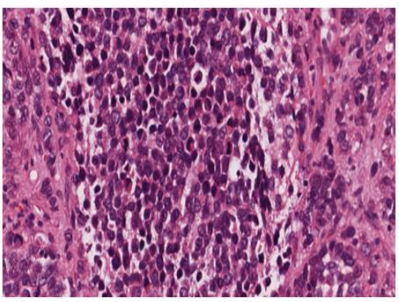

Fig. 2. Embryonal rhabdomyosarcoma consisting of small and blue cells with desmoplastic stroma H/E 10X

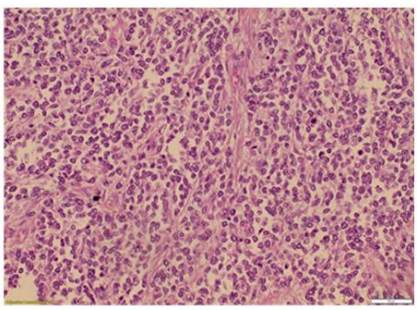

Fig. 3. Embryonal rhabdomyosarcoma consisting of small and blue cells. H / E 20 X

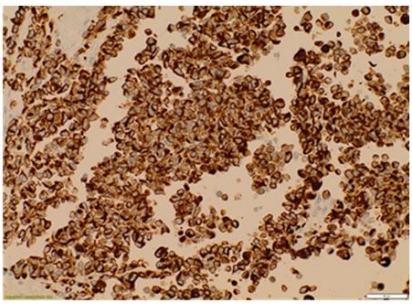

Fig. 4. Embryonal rhabdomyosarcoma. Neoplastic cells showing positivity for Desmin. IHC 20X

Anaplasia is a negative prognostic factor, regardless of whether it is focal or diffuse [3,13]. Anaplasia specifically requires multipolar mitotic figures. Make sure you are not overinterpreting degenerating or apoptotic cells.

Look for organization of the division and exclude cells that appear to be "exploding". Also try to avoid interpreting overlapping cells as one larger cell Extremity involvement is associated with more relapses and lower survival.

Pure embryonal RMS account for about 49% of all RMS, affecting mostly children under 10 years old, although, they also occur in adolescents and young adults with much less frequency, being rare in adult patients [6]. The most common histologic type is embryonic, as in our case [7].

Primary testicular mesenchymal tumors may originate within testicular teratomas or from interstitial testicular stromal cells or multipotential primitive cells with a capacity of multiple differentiations. In our case, there was no evidence of association with testicular teratoma, which is why we conclude it as a primary sarcoma of intraparenchymal location.

Among the sarcomas that has been registered as primary testicular they have been reported, osteosarcomas, leiomyosarcomas, Kaposi's sarcoma, rhabdomyosarcoma [7,9,10,14-20].

The clinical stage of these tumors is very variable and depends on the location, age of the patient and stage at diagnosis, with or without the presence of distant metastases. A reasonable number of these tumors in early stages are asymptomatic, so in most cases they are diagnosed in advanced stages. The survival rate of patients with these tumors has undergone a significant increase in the last decades, going from a 5-year disease free survival from 66% to 74% in the last 20 years [14].

The diagnosis of these sarcomas is based on histopathological and immunohistochemical studies. In the histological study we can see a diverse

morphology, from RMS with poorly differentiated pattern difficult to diagnose without IHC study, and other well differentiated that resemble the fetal muscle [6,7,13]. The patient reported in this study presented a poorly differentiated pattern in its totality, which required IHC studies for the conclusive diagnosis.

From the microscopic point of view, we can observe variable degrees of cellularity, with hyper cellular areas alternating with myxoid areas, presence of different types of cells with variable proportion from one tumor to another: undifferentiated round cells, spindle cells and rhabdomyoblasts. The stroma is usually collagen with variable amounts of myxoid material and in a percentage of cases transverse striations are observed in the cytoplasm of the tumor cells [6,21].

The microscopic aspect of the reported case corresponds to a poorly differentiated tumor, formed mostly by small, round cells with hyper chromatic nuclei, poorly defined cytoplasm, little variation in cell size, abundant mitosis, without the presence of rhabdomyoblasts, or fusocellular component.

Several immunohistochemical stains were performed for the differential diagnosis of small and blue cell tumors; as well as studies of germ cell tumor markers, to rule out its origin (Table 1).

Table 1. Result of Immunohistochemistry

Marker	Results
Vimentin	Positive
Desmin	Positive
Enolase	Negative
S100 Protein	Negative
CD99	Negative
LCA	Negative
Muscle-Specific Actin	Negative
Alpha-fetoprotein	Negative
PLAP	Negative
HCG	Negative

The case presented positive immunoreactivity for vimentin and desmin, being negative for the rest of the markers, including the muscle specific alpha-actin. Many markers were applied for the diagnosis of RMS, but its usefulness, sensitivity and specificity vary. Among the most used we find the desmin that marks the intermediate filaments of the muscle, it is positive in more than 90% of the RMS although it can be positive in 50 to 70% of the leiomyosarcomas. Its positivity has been reported for some small round and blue cell tumors as is the case of Ewing's Sarcoma/Primitive Neuroectodermal tumor [6,22,23].

Although, myoglobin is a specific marker for skeletal muscle tumors, it is not very sensitive [6]. The positivity of this marker is manifested more in better differentiated cells.

Immunostaining with my regulatory proteins such as MyoD1 have a high sensitivity and specificity [6,14,20,24]. Although the expression of MyoD1 is higher in the alveolar RMS than in the embryonal. This marker has high utility for the differential diagnosis of small and blue cell tumors, RMS and between pleomorphic RMS, and other pleomorphic sarcomas. In our case the diagnosis was based on the morphology of a tumor of round, small and blue cells, so a mobile directed for this purpose was indicated and to rule out the association with germ cell tumors of the testicle. The immunophenotype of the case studied showed positivity only for vimentin and Desmin, with a null expression for lymphoid, neuroectodermal, smooth muscle, epithelial, and germ cell markers (Table 1). The placental alkaline phosphatase (PLAP) that marks germ cell tumors have been positive in some rhabdomyosarcoma, especially alveolar type [6,7,24]. In our case it was negative for this marker.

The alveolar RMS is associated with a frequent translocation, t (2; 13) and a less common t (1; 13), which results in a fusion of the PAX3 and PAX7 genes, respectively, with the FKHR (fork head in rhabdomyosarcoma) located at 13q1431. There are no markers of this type, for the rest of the RMS [24,25].

Fusion-negative cases (FN) of histologic ARMS share clinical and genomic features of ERMS, such as loss of heterozygosity (LOH) at chromosome 11p15.5. ERMS is also characterized by specific recurrent chromosomal gains and losses, affecting cell cycle genes. In line with these findings, future classification of RMS will be based on fusion status, classifying rhabdomyosarcomas as either fusion positive (FPRMS) or fusion negative (FNRMS).

Regarding the prognosis of RMS, in the last decades it has improved, before the 60s the prognosis was poor with a 5-year-old average survival. This has been enhanced with the multidisciplinary management consisting of biopsy or radical surgery according to the location, combined chemotherapy with or without radiotherapy [7].

In our case, once the diagnosis was confirmed and once the extension study was completed, the treatment was carried out following the current SIOP protocol in our center.

Favorable and unfavorable factors are described in rhabdomyosarcomas [6,26]. In our case, favorable factors include age, location, absence of regional and distant metastases, and complete initial resection. Among the unfavorable is the tumor size that was greater than 5 cm.

Mitotic activity has little value in predicting response to treatment and disease progression, as well as studies of ploidy that are not yet conclusive [6]. Recurrence is seen with frequencies in inadequately treated patients [27,28].

3. CONCLUSIONS

The RMS is the most frequent soft tissue tumor in childhood and the genitourinary location is the second most frequent after the head and neck region. Primary intratesticular rhabdomyosarcoma is uncommon. The diagnosis is made by histopathological study supported by Immunohistochemistry. With the new treatment strategies, the survival of patients affected with this tumor has improved remarkably.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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This chapter is an extended version of the article published by the same author(s) in the following journal.
Clinical Oncology: Case Reports, 1(1): 01-04, 2018.

Chapter 4
Print ISBN: 978-93-5547-742-2, eBook ISBN: 978-93-5547-749-1

Oral Myeloid Sarcoma

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DOI: 10.9734/bpi/cpms/v9/3498B

ABSTRACT

The terms "chloroma," "myeloid sarcoma," and "granulocytic sarcoma" (GS) all refer to a terrible subtype of myeloid leukaemia. Acute Myeloid Leukemia (AML) is a hematologic cancer that can occasionally colonise extramedullary locations like the skin, gingiva, central nervous system (CNS), orbit, liver, or, in rare cases, the tongue, leading to a variety of appearances. Here, we discuss the case of a 30-year-old man who arrived with hepatomegaly and a tongue ulcer that appeared to be malignant. After examination, it was determined that it was a myeloid sarcoma of the tongue and liver, which ultimately revealed an underlying AML.

Keywords: Acute myeloid leukemia (AML); myeloid sarcoma (MS); granulocytic sarcoma (GS).

1. INTRODUCTION

According to the most recent classification by the World Health Organization (WHO), granulocytic sarcoma, also known as myeloid sarcoma (MS), is a tumoral lesion made up of immature cells that have a green colour that is caused by the enzyme myeloperoxidase (MPO). It has been recognised as an acute leukaemia extramedullary presentation, particularly in acute myeloid leukaemia. It was demonstrated that it could be seen both at the onset of the disease and during its progression, as well as at relapses following allogeneic stem cell transplantation. Less commonly, it has been observed during the course of myelodysplastic syndrome, chronic myeloid leukaemia and other myeloproliferative diseases Soft tissue changes in the oral cavity such as oral bleeding, mucosal petechiae, gingival enlargement, mucosal ulceration, necrosis, and infection, are known to be associated with leukemia [1,2,3]. The infiltrations of leukemic cells within the oral cavity is rare. However, some case reports on leukemic cells infiltrating tissues of the oral cavity, including the gingiva were reported [4]. The ulcers may affect any part of the oral mucosa, including the tongue and palate [5].

The objective of this report is to discuss a case of leukemic infiltration of the oral cavity and liver of a 30year-old male patient. He had a rare clinical presentation

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characterized by the development of persistent ulceration on the right lateral border of the tongue and hepatomegaly. We report the clinicopathological and immunohistochemical characteristics of this malignancy.

2. CASE

A 30-year-old chronic tobacco chewer presented to oncology OPD with a chronic non-healing ulcer in the right lateral border of the tongue and abdominal discomfort. On examination it was 2*1 cm in size, irregular in out line with infiltrating borders covered by yellowish grey necrotic slough in right lateral border of tongue and hepatomegaly (liver span -18 cm). Patient was admitted for a baseline workup and punch biopsy (Fig. 1).

Fig. 1. Right lateral border of the tongue showing a hardened and ulcerated lesion

He had a hemoglobin of 7.9 g/dl, total leucocyte count was 96,230 cells/ul and platelets were 3, 73,000 cells/ul without any atypical cells in the peripheral blood smear. His base line renal and kidney function was normal. A Punch biopsy was done which showed acanthotic epithelium with focal ulceration and granulation tissue with medium to large atypical cells with eosinophilic cytoplasm, hyperchromatic nucleus and prominent nucleoli infiltrating the muscle bundles. It was reported as granulocytic sarcoma (Fig. 2).

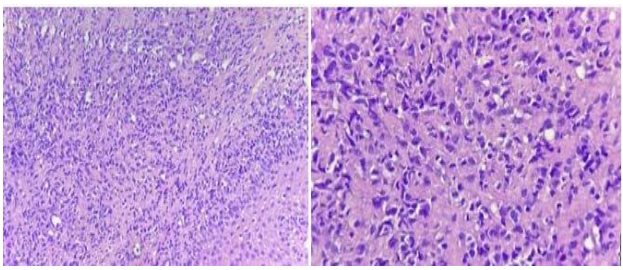

Fig. 2. Photomicrographs of the tongue showing stratified squamous epithelium with sub epithelium showing diffuse infiltrate of monocytoid cells. (H&E) IHC was done on the specimen which showed MPO positive, CD 117 occasional positive, CD 15 & CD 34 were negative and a granulocytic sarcoma was favored (Fig. 3)

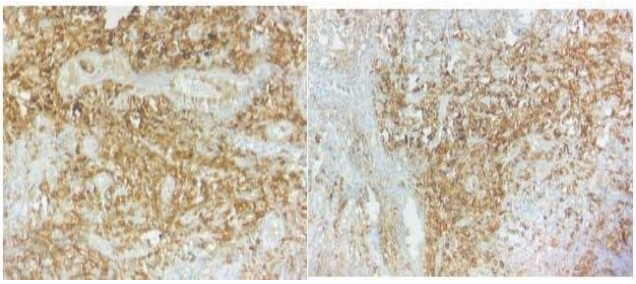

Fig. 3. Immunohistochemistry (DAB; 200x). Cytoplasmic Positivity for MPO

A bone marrow aspirate was done on this patient it showed AML M5a variant (Fig. 4). A flow cytometric analysis was done on the bone marrow specimen and it revealed 85% blasts were gated using CD45 V500c vs. side scatter. The blasts mainly expressed myeloid markers MPO, CD13, CD33, CD14, CD15 and CD117 along with HLADR and CD34 suggestive of AML M5a.Karyotyping was showed normal karyotype. Genetic profile including BCR-ABL, inv16, t(8:21) mutation done by FISH (fluorescence in situ hybridization) and FLT3 ITD/D835 mutation by PCRRFLP were negative.

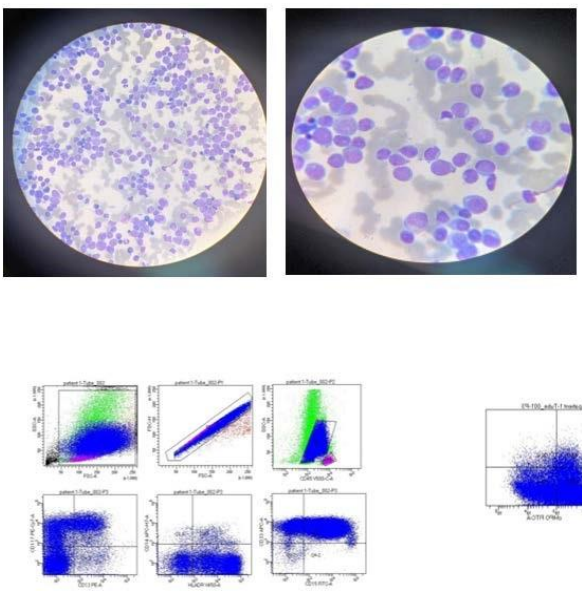

Fig. 4. Acute Monoblastic leukemia (AML M5) with flow cytometry showed bivariate dot plots illustrating abnormal blasts (blue) with the expression of myeloid (MPO, CD13 and CD33) and monocytic markers (CD14, CD15, CD117, CD34, and HLA-DR)

CT scan was suggestive of Presence of few hypodense lesions noted in both lobe of liver, largest measures 28x26 mm in segment VII of liver s/o infiltration (Fig. 5).

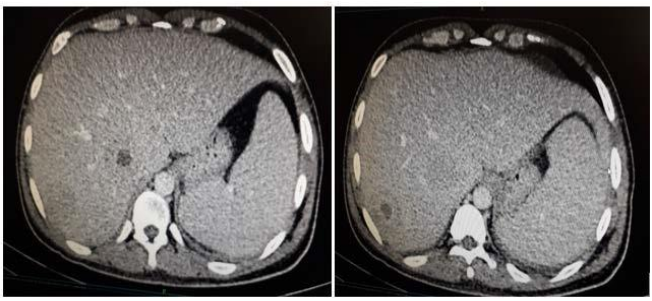

Fig. 5. CT scan showing hypodense lesions in liver Biopsy of liver was performed; Section shows normal liver parenchyma, mixed inflammatory infiltrate along with immature and mature granulocytic precursor's cells. In a known case of Acute Myeloid leukaemia (AMLM5), infiltration by same is suggested Granulocytic sarcoma (Fig. 6)

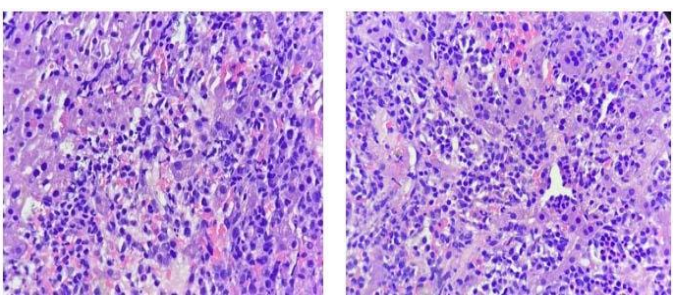

Fig. 6. Liver biopsy shows infiltrate of monocytoid cells in to the parenchyma with destruction of normal architecture

Patient was treated with HIDAC (High dose cytarabine) 2 GM/M2 for 2 days in view of poor performance status and patient developed fever on day3 so started on broad spectrum antibiotic and chemotherapy was stopped. Patient had persistent leukemic activity on day 20 of HIDAC chemotherapy. Patient was received standard induction chemotherapy 7+3 consists of 7 days of standard-dose cytarabine (100 mg/m2), and 3 days of daunorubicin (60 mg/m2). Clinical response in form of disappearance of tongue ulcer on day 18 of induction chemotherapy. Patient achieved complete remission in bone marrow after induction chemotherapy and received four cycles of high-dose cytarabine (3 g/m^2, bid, Days 1,3,5) consolidation chemotherapy.

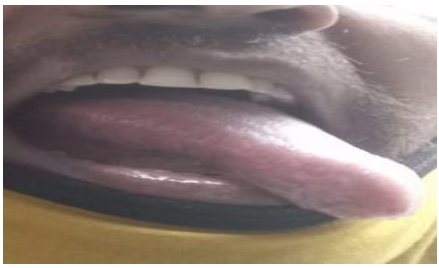

Fig. 7. Clinical appearance of the tongue after the first cycle of chemotherapy

3. DISCUSSION

Leukemia is a worldwide public health problem. The microscopic and molecular diagnostic criteria, currently proposed by WHO [1] for its classification, require the molecular markers. The rate of new cases of acute myeloid leukemia was 4.3 per 100,000 men and women per year. The death rate was 2.8 per 100,000 men and women per year. Estimated New Cases 2021 are 20,240 and Estimated Deaths 2021 are 11,400. Acute myeloid leukemia represents 1.1% of all new cancer cases in the U.S. 2 [1-7].

Myeloid sarcoma is a rare extramedullary tumor formed with immature myeloid cells. The clinical course of granulocytic sarcoma can be varied and may be associated with three clinical situations: (i) AML, (ii) chronic myeloproliferative disorders, or (iii) Myelodysplastic syndrome [8]. Isolated myeloid sarcomas are unusual and typically progress to form AML [1]. The clinical features reported on the oral cavity are diverse. The most common presentation of the tumor is a nodule with variables pigmentations, possible ulceration, and bleeding. It can affect the oral cavity on the tonsils, lips; gingiva, palate and tongue [5]. Among the different acute leukemia, the myelocytic and monocytic variants most frequently cause severe oral changes [4].

The oral manifestation of myeloid sarcoma is rare. To the best of our knowledge, few cases have been reported in the literature, of which some cases had isolated oral myeloid sarcomas with bone marrow involvement [9]. According to Stafford et al., [9] oral lesions are more frequently seen in patients with acute leukemia. Oral manifestations are three times less frequent in chronic leukemia compared to acute leukemia [3]. They may be either the result of direct infiltration by leukemic cells (primary), or could be secondary to underlying thrombocytopenia, neutropenia, or impaired granulocyte function. Gingival infiltration, as the initial presentation of AML, is seen in 5% of the cases. Dreizen et al. [10] showed that the incidence of gingival infiltrates was higher in patients with acute monocytic leukemia (66.7%), followed by those with acute myelomonocytic leukemia (18.5%) and acute myeloblastic leukemia (3.7%).

As seen in our case, in the majority of the patients, myeloid sarcoma occurs in association with AML. However, Byrd et al. [11] showed that the granulocytic sarcoma could be initially misdiagnosed in 46% of leukemia patients. Our case highlights a rare clinical presentation of myeloid sarcoma in the oral cavity in the context of AML, characterized by the development of persistent ulceration on the lateral border of the tongue, in addition to the hepatomegaly.

The cytogenetic analysis is one of the most important prognostic determinants in AML [12]. Based on the cytogenetics and molecular findings, patients are stratified as having favourable, intermediate, and unfavourable risk [13].

Our case revealed no mutations in FLT3 and NPM1 genes, patient stratified as intermediate risk.

Recently, Visani et al. [14] stated that the combination of genetic, epigenetic, and transcriptional data would represent the molecular basis for treatment decisions with the highest predictive potential. These agents include FLT3 inhibitors, farnesyl-transferase inhibitors, histone deacetylase inhibitors, and DNA methyltransferase inhibitors Although the infiltration of malignant cells into the oral tissues is not an uncommon feature in leukemic patients, especially in patients with acute leukemia, infiltration of the tongue is rare . On the other hand, while myeloid sarcomas can occur in any part of the body, the involvement of the oral cavity is uncommon, with only 37 cases reported according to the Yap et al. [15] report. Another recent case-report by Ignacio-Cconchoy et al. [16] also described oral myeloid infiltration in the tongue as the first manifestation of an oncohematologic disease. Lillington et al., the patient was diagnosed to have a GS with a normal marrow. However, the cells in marrow as well as the GS had the same molecular and cytogenetic abnormality highlighting presence of occult myeloid disease despite a normal marrow. This indicates that isolated GS and GS could be in the same disease spectrum [17]. The most common differential diagnosis for a GS is a lymphoma followed by malignant melanoma, undifferentiated cancer, and extramedullary hematopoiesis [18].

We present a case of a myeloid sarcoma of the upper vestibular gingiva in a 29- year- old woman who has no hematologic disease history. Multiple metastases were found in floor of the nasal cavity, left breast, and left lacrimal gland 12 months after primary diagnosis [19].

Rare case of monoblastic sarcoma in a 50-year-old male patient without a history of acute myeloid leukaemia with involvement of the paraspinal region, abdominal wall, and tongue [20].

Modalities of treatment are radiotherapy, surgery or chemotherapy. Although there is no important role for surgery for symptomatic MS patients, excision or debulking may be considered before starting chemotherapy [21]. While there is no consensus on the treatment of MS, it is almost always treated with AML induction regimens. In the literature, since there is a high rate for progression to acute leukaemia especially in patients who are treated with localized methods

(88100%), systemic treatment is recommended to all patients with isolated MS. The main role of surgery becomes clear when reaching a diagnosis is difficult and an excisional biopsy is needed. Radiotherapy should be considered in isolated MS, inadequate response to chemotherapeutic regimen, in recurrence following bone marrow transplantation, and when rapid symptom relief is needed. Using a regimen of 24 Gy in 12 fractions can be offered to most MS patients [22].

4. CONCLUSION

MS is considered to be a subgroup of AML where myeloid blasts form a tumor mass occurring at a site other than the bone marrow.

The diagnosis of myeloid sarcomas in the oral cavity and liver can be very challenging, especially without a history of hematological disorders or gingival leukemic involvement, due to nonspecific clinical features. These patients offer insights to the pathophysiologic, diagnostic, and therapeutic challenges associated with this rare disorder. A sound knowledge of the disease for a clinician and an expert pathologist is an utmost necessity for early suspicion, diagnosis, and treatment of MS.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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This chapter is an extended version of the article published by the same author(s) in the following journal.
International Journal of Medical Sciences and Innovative Research, 7(2): 15–20, 2022.

Chapter 5
Print ISBN: 978-93-5547-742-2, eBook ISBN: 978-93-5547-749-1

Methyl-donors Induce Apoptosis and Attenuate Proliferation Pathways Mediated by Akt and Erk1/2 in Breast and Lung Cancer Cell Lines

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DOI: 10.9734/bpi/cpms/v9/2576A

ABSTRACT

We aimed to explore how methyl-donor treatments affect the growth, proliferation, apoptosis and the related pathways in hormone positive invasive breast cancer (MCF7 and T47D) and NSCLC lung cancer (A549 and H1650) cell lines**.** Methyl-donors are employed as an adjunctive support in oncotherapy and play critical roles in physiological processes catalyzed by coenzymes of the B vitamins. Our theory was that methyl-donors may directly inhibit tumor development and proliferation in addition to helping patients tolerate cancer treatment. Methyl-donor treatment significantly reduced the proliferation in all investigated cell lines, possibly through the downregulation of MAPK/ERK and AKT signaling. These were accompanied by the upregulation of the pro-apoptotic Bak, Bax both in MCF7 and H1650 cells, at reduced anti-apoptotic Mcl-1 and Bcl-2 levels in MCF7 and H1650 cells, respectively. The treatment induced downregulation of p-p53(Thr55) was likely to contribute to protecting the nuclear localization and apoptosis inducing functions of p53. The features that are being offered are known to increase the sensitivity of cancer treatment. In light of this, the observations are consistent with the theory that methyl-donors may protect p53 activities by promoting apoptotic signaling by downregulating both the MAPK/ERK and the AKT pathways in breast and lung cancer cell lines. Our results can emphasize the importance and benefits of the appropriate dietary supports in cancer treatments. However, further studies are required to confirm these effects without any adverse outcome in clinical settings**.**

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Keywords: Breast cancer; lung cancer; methyl-donors; apoptosis; complementary therapy.

1. INTRODUCTION

Complementary care, such as adequate diet, psychological support, and physical activity, plays a larger impact in the success of cancer treatment than previously thought [1-7]. Elevated dietary methyl-donor intake, for instance, has been suggested to contribute to cancer prevention by reducing the risk of breast [8-10], lung [11] and colorectal cancers [12,13]. However, the mechanisms behind this has not been fully clarified, which attracted our attention to study the effects of methyl-donors in cancer. Recent advances in high throughput DNA sequencing and single cell DNA methylation analysis have revealed existence of distinct epigenetic signatures in variety of cancer types and the extent of epigenetic changes is correlated with tumor stage and type [14].

Dietary methyl-donors including folate, betaine, choline, methionine, and other B vitamins like B2, B6, and B12 perform crucial physiological roles such amino- and nucleic acid metabolism, redox defense, cell development, and apoptosis [1]. These components provide methyl groups for the one-carbon metabolism, including the methionine and folate cycles, besides being involved in the trans-sulfuration methylation pathways [2]. Inadequate DNA methylation may lead to the development of cancer, mainly by the shortage of the necessary vitamins and minerals for the proper functioning of these pathways [3]. Indeed, a recently published meta-analysis revealed that folate is associated with decreased risk of all-cause mortality and a wide range of chronic disease [15]. Another review reported similar results, where low or deficient folate status was associated with several cancers, except prostate cancer, which prevalence was linked with folic acid (FA) supplementation and higher serum level [16]. The tight interrelationship between choline, methionine and folate has already been known from in vivo studies, which recommend the testing of all three agents, when studying diet and DNA methylation [17]. The folate-mediated one-carbon metabolism (FOCM) is highly sensitive to nutrition status of several B vitamins as well, that alter network outputs [18]. Additionally, a majority of clinical and randomized clinical trials have shown that of methyl-donor micronutrient intake affects DNA methylation by reducing the risk of several cancer types [3]. Furthermore, folate can induce apoptosis via PTEN/AKT/P53 signaling pathway, and through reducing the effects of both the AKT and ERK signalling in breast cancer [19,20]. It is known, that AKT signaling can inhibit the activation of the pro-apoptotic Caspase-9 and Caspase-3 [20,21], and that upstream inhibitors of the MAPK pathway can reduce tumor proliferation and mediate apoptosis, though Erk1/2 activation may also be involved in apoptosis induction [22]. Interestingly, a DNA methylation reader methyl-CpG-binding protein 2 (MeCP2) regulates genes linked to tumorigenesis as well [23], and promotes proliferation via activation of Erk1/2, and its loss of function induces apoptosis [24]. Natural compounds and green tea polyphenols are able to downregulate MeCP2 [25,26]. B vitamins are also required for the energy-yielding metabolism, oxygen transport and neuronal

functions. Therefore, they are essential in cognitive and psychological functions, including mental and physical fatigue [5,6].

Breast cancer incidence is still growing worldwide, although the 5-year relative survival rate improved around 10% through the last 40 years, where prevention and the early diagnosis contributed to almost half of this reduction even in breast cancer mortality [7]. Lung cancers account for the most cancer-related deaths worldwide [27]. The larger proportion of these cases are non-small cell lung cancers (NSCLC), which majority are at advanced stages when diagnosed, thus they frequently develop resistance to the first or second generation targeted therapies [27]. Pancreatic cancer is an aggressive malignancy with high metastatic potential. There are several lifestyle-related determinants in its etiology, including diet. Methyl donors are dietary micronutrients which play an important role in fueling vital metabolic pathways, and as bioactive food components provide methyl groups as substrates and cofactors. The imbalanced nutritional status of methyl donors has recently been linked to pathological conditions [28,29].

Cancer treatments primarily focus on the elimination or at least the reduction of tumor burden and on preventing cancer spreading to distant locations from the primary sites. However, almost equally important is to keep up both the mental and physical energy and motivation of the patients, which are required for their life activities as close to normal as possible, despite the trauma they are going through. To achieve this, it is important to support the sensitivity and reduce the side effects of cancer treatments, for their optimized efficiency. Methyl-donors, such as folate and cobalamin (B12) support of pemetrexed-based chemotherapy treated lung cancer patients, or pyridoxine (B6) and B12 as well as thiamin (B1), has only been applied in the recent treatment regimens to reduce the symptoms of the side effects induced by systemic chemotherapy [30-32]. Evidence-based complementary therapies, including dietary support, could greatly assist in reaching both of the above mentioned targets [6,33].

In this work, we aimed to explore how methyl-donor treatments affect the growth, proliferation, apoptosis and the related pathways in hormone positive invasive breast cancer (MCF7 and T47D) [34] and NSCLC lung cancer (A549 and H1650) cell lines.

2. RESULTS

2.1 Methyl-donors Affect Tumor Cell Proliferation

We used the MTS proliferation assay to detect the effects of methyl-donor treatments on cancer cell growth both in breast cancer and lung cancer cell lines. The highest concentration of methyl donors significantly decreased the proliferation rate in all cell lines (48h and 72h at MCF7 $p < 0.05$ and $p < 0.01$, respectively, 24h at A549 $p < 0.01$, 72h at T47D $p < 0.01$, and 72h at H1650 $p < 0.001$) compared to the non-treated control.

2.2 The Effects of the Methyl-donors on the Cell Cycle

Treatment related changes in the cell cycle and apoptosis were tested using flow cytometry. The subG1 fractions, indicating also the apoptotic cells, was increased in all methyl-donor treated cell lines. The changes were significant only in the breast cancer ($p < 0.001$ at both timepoints in MCF7, $p < 0.01$ in T47D cells) (Fig. 1A-F), and A549 lung cancer (Fig. 2A, C), but not in the H1650 cell lines (Fig. 2B, D). However, inverse tendency of changes in the subG1 vs G1 phase fractions, i.e. increase vs decrease, respectively, were seen in all cell lines.

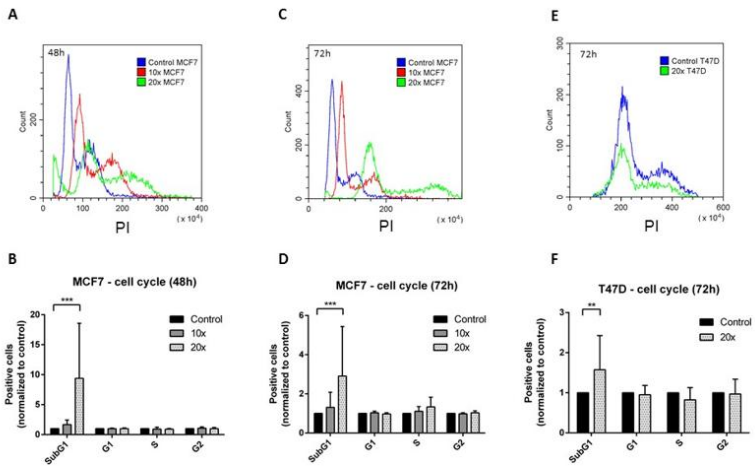

Fig. 1. Cell cycle analysis of the MCF7 and T47D cells. SubG1 fraction of breast cancer cell lines were significantly increased after methyl-donor treatments compared to untreated controls. SubG1 fraction increased significantly in MCF7 cells both after 48h (A-B) and 72h (C-D), and in T47D cells after 72h (E-F) treatments. Each bar represents the average number of positive cells normalized to control from at least 3 repeats ± SD. Statistical significance: **: $p < 0.01$ in T47D; ***: $p < 0.001$ in MCF7 cells. 10x and 20x: concentrations of methyl-donors

2.3 Detection of Apoptosis and Related Pathway Elements after Methyl-donor Treatments

Significantly elevated number of Annexin-V single positive, early apoptotic cells were detected in T47D breast cancer ($p < 0.01$) by flow cytometry after methyl-donor treatments compared to controls (Fig. 3A), however only a tendency ($p = 0.41$) of increase was seen, and only at 48h (Fig. 3B), but not at 72h in MCF7 cells. Moreover, significantly elevated early apoptotic cells were detected both in A549 and H1650 lung cancer cell lines ($p < 0.001$ and $p < 0.05$, respectively) (Fig. 4A-B).

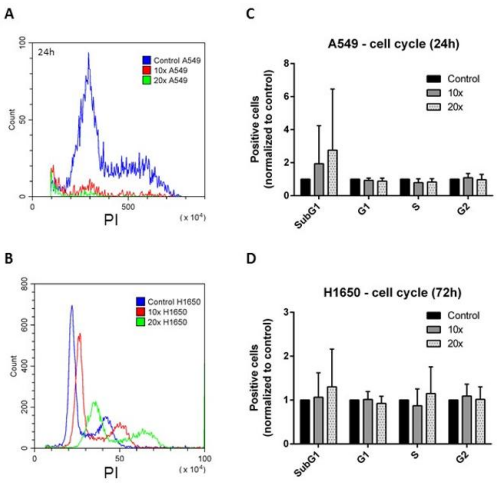

Fig. 2. Cell cycle analysis of A549 and H1650 cells. Only a tendency of increased SubG1 fractions were seen in lung cancer cell lines A549 and H1650 (p = 0.35 and p = 0.46, respectively). 10x and 20x: concentrations of methyl-donors

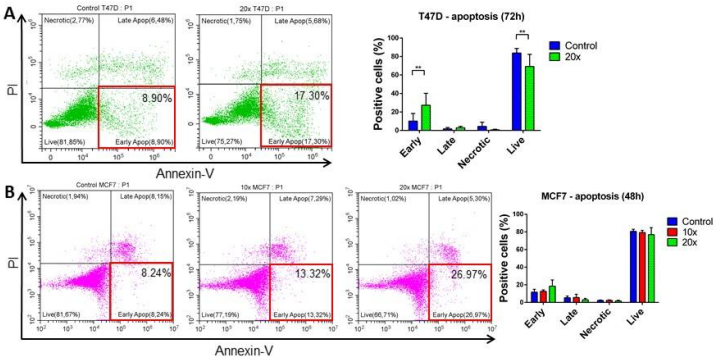

Fig. 3. Apoptosis detection in T47D and MCF7 cells. Early apoptotic cells (Early; red square highlighted areas, lower right squares) of T47D were increased significantly (A) compared to controls after 72h methyl-donor treatments, while MCF7 showed only a tendency of increase after 48h (B). Each bar represents the average percentage of positive cells in early apoptotic, late apoptotic, necrotic and live cells area from at least 3 repeats ± SD. Statistical significance were plotted as **: p < 0.01. Late: late apoptotic cells; Necrotic: necrotic cells; Live: live cells. 10x and 20x: concentrations of methyl-donors

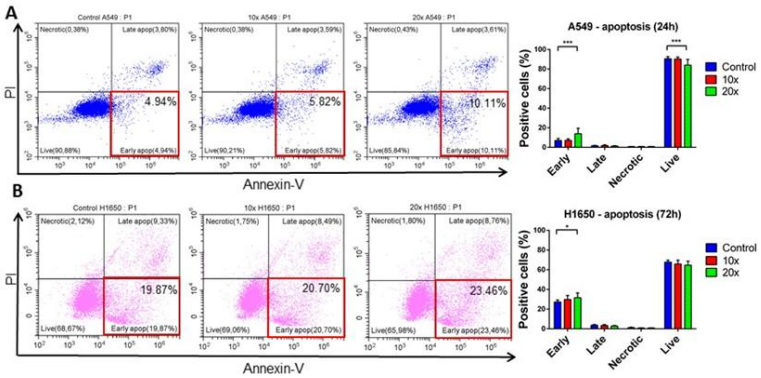

Fig. 4. Apoptosis detection in A549 and H1650 cells by flow cytometry. Early apoptotic cells (red squared highlighted areas, lower right squares) were significantly elevated at the highest concentration of methyl-donor treated A549 and H1650 cell lines after 24h and 72h, respectively, compared to control. Each bar represents the average percentage of positive cells in early apoptotic, late apoptotic, necrotic and live cells area from at least 3 repeats ± SD. Statistical significance were plotted as *: $p < 0.05$; ***: $p < 0.001$. Early: early apoptotic cells; Late: late apoptotic cells; Necrotic: necrotic cells; Live: live cells. 10x and 20x: concentrations of methyl-donors

Furthermore, we investigated the methyl-donor induced changes in the expression of pro-, and anti-apoptotic proteins using Western blot. The dynamism of changes varied at different time points and methyl-donor concentrations. In MCF7 breast cancer cells the intrinsic apoptotic pathway induced Caspase-9 and the pro-apoptotic Bak, Bax protein levels were significantly increased at 72h ($p < 0.033$, $p < 0.033$, and $p < 0.001$, respectively), in line with the significant reduction of the anti-apoptotic Mcl-1 protein ($p < 0.033$). However, the anti-apoptotic Bcl-2 levels were elevated both after 48h and 72h ($p < 0.033$ and $p < 0.002$, respectively), and the pro-apoptotic Puma levels were decreased significantly ($p < 0.033$). These changes occurred mostly when the higher i.e. x20 concentration of methyl-donors were used (Fig. 5A). In T47D lung cancer cells neither the pro-apoptotic, nor the anti-apoptotic proteins changed significantly after treatment (Fig. 5B).

In H1650 lung cancer cell line, the pro-apoptotic Caspase-9, Bak, Puma, Bax protein levels were elevated, while the anti-apoptotic Bcl-2 levels decreased significantly at unchanged Mcl-1 levels, after methyl-donor treatment. All significant changes were seen at both methyl-donor concentrations, expect in case of Caspase-9, where only the lower concentration resulted in significant increase (Fig. 6A). In A549 cells, only the increase of Caspase-9 levels was significant ($p < 0.002$), but Bak and Mcl-1 also showed a strong tendency of increase and reduction, respectively ($p = 0.10$ and $p = 0.15$, respectively) (Fig. 6B).

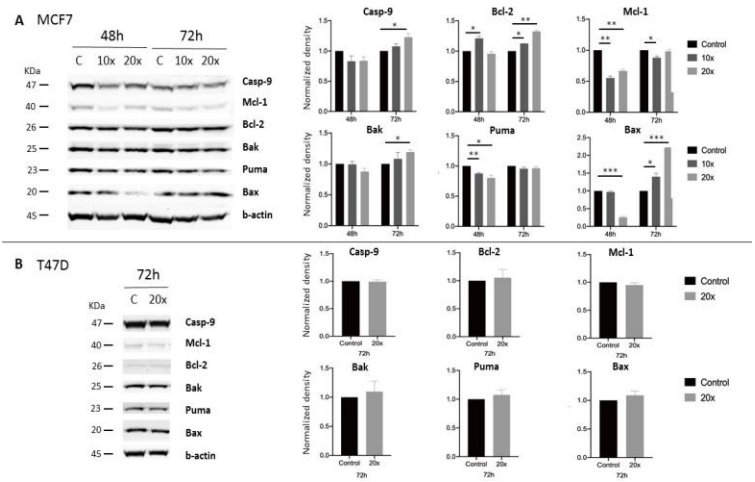

Fig. 5. Western blot analysis of apoptotic proteins in methyl-donor treated MCF7 and T47D cell lines. Pro-apoptotic Caspase-9, Bak and Bax were significantly increased in MCF7 cells after 72h treatments, while the anti-apoptotic Mcl-1 significantly diminished. Interestingly, the pro-apoptotic Puma and the anti-apoptotic Bcl-2 changed significantly in an opposite way as expected (A). In case of T47D none of the changes were significant (B). Each bar represents the average normalized density from at least 3 repeats ± SD. Statistical significance are plotted as *: $p < 0.033$, **: $p < 0.002$, or ***: $p < 0.001$. C: control; Casp-9: Caspase-9. 10x and 20x: concentrations of methyl-donors

2.4 Investigating the MAPK/ERK and AKT Signaling Pathways

Then, we tested how methyl-donor treatments affect major growth and proliferation related pathways by focusing on Akt, and p-p44/42 MAPK (p-Erk1/2) expression. We also explored the activation and expression of p53 protein through detecting its forms phosphorylated either at Ser15 or Thr55.

Methyl-donor treatment significantly reduced Akt in MCF7 at 48h, T47D and A549 (Fig. 7A-D, E), as well as the p-Erk1/2 protein levels in MCF7 at 48h, T47D, A549 at 20x concentration and in H1650 (Fig. 7A-D, F) compared to the controls. However, pan Akt as well as the p-Erk1/2 increased at 72h in MCF7 cells, and Akt in H1650 cells in the same manner as in MCF7. Additionally, p-Erk1/2 in A549 cells when using the lower treatment dilution (Fig. 7E-F). Phosphorylation of p53 at Ser15 was significantly increased only in MCF7 cells after 72h at both methyl-donor dilutions (Fig. 7A-D, G). Activated p53 (Thr55) was significantly decreased in all cases as a result of the methyl-donor treatments (Fig. 7A-D, H). Neither p-Akt, nor p21 expression could be detected in any cases, so as p53 (Ser15) in A549 cells.

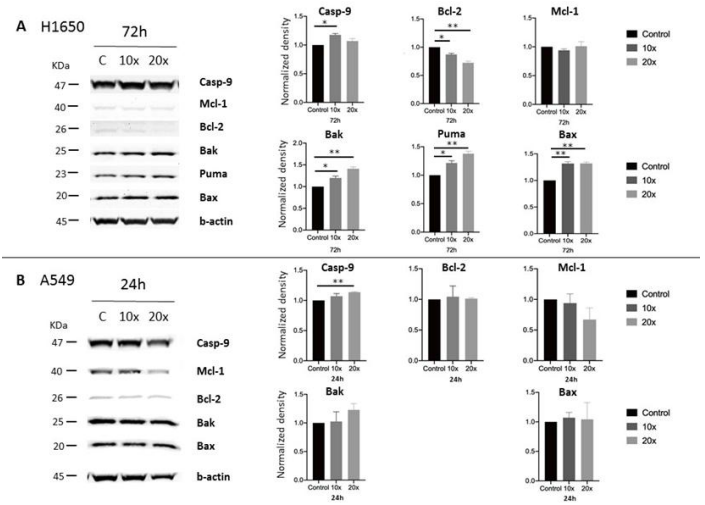

Fig. 6. Western blot analysis of apoptotic proteins in methyl-donor treated A549 and H1650 cell lines. Clear opposite changes of pro-apoptotic vs. anti-apoptotic proteins were seen in both lung cancer cell lines. In H1650 cells all protein changed significantly after the treatments, except the Mcl-1 (A). In A549 only the Caspase-9 increased significantly (B). Each bar represents the average normalized density from at least 3 repeats ± SD. Statistical significance are plotted as *: p < 0.033, **: p < 0.002. C: control; Casp-9: Caspase-9. 10x and 20x: concentrations of methyl-donors

3. MATERIALS AND METHODS

3.1. Cell Culture Conditions

Breast (MCF7, T47D) and lung cancer (A549, H1650) cell lines were purchased from ATCC (Manassas, VA, USA), and DSMZ (Braunschweig, Germany), and used for in vitro experiments. MCF7 human breast adenocarcinoma (HTB-22) and H1650 lung adenocarcinoma (CRL-5883) cell lines were cultured in RPMI1640 (LM-R1640; Biosera, Nuaille, France). A549 lung carcinoma (CCL-185) cell line was grown in Dulbecco's Modified Eagle Medium (DMEM with 4.5 g/l glucose, BE12-604Q; Lonza, Basel, Switzerland). T47D human ductal carcinoma (HTB-133) cell line was maintained in Ham's F12 Nutrient Mix (21765-029; Thermo Fisher Scientific, Waltham, MA, USA).

All media were supplemented with 10% fetal bovine serum (FB-1090; Biosera, Nuaille, France) and 0.4% gentamycin (Sandoz, Basel, Switzerland; 80 mg/2 ml). For T47D, 10 µg/mL insulin (12585-014; Thermo Fisher Scientific, Waltham, MA, USA) also was added to medium. All of the cell lines were kept under standard culture conditions (5% CO_2, 37°C).

All cell lines are regularly tested for Mycoplasma sp. applying a PCR test following methodological article written by Uphoff et al. [35]. Only negative cell lines are used for research purposes.

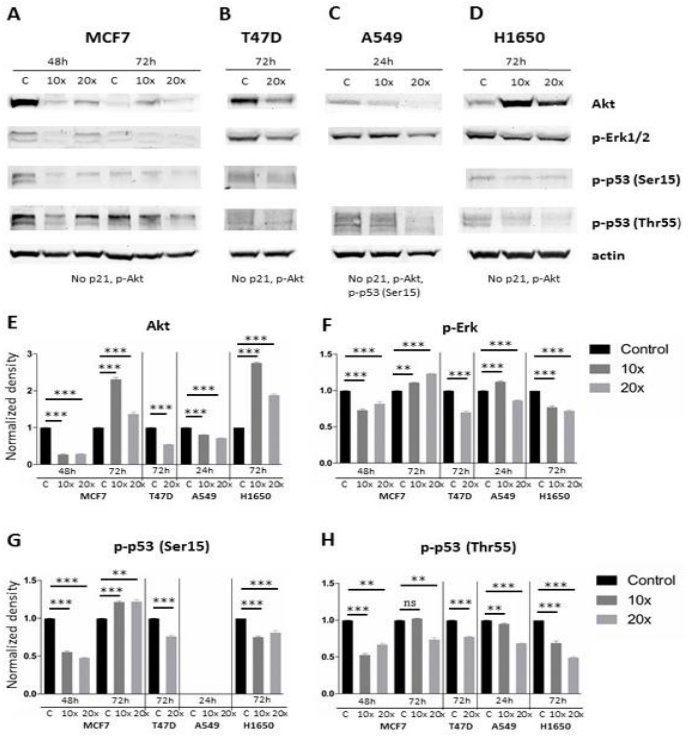

Fig. 7. Changes of the level of selected signaling proteins after methyl-donor treatment in MCF7, T47D, A549 and H1650 cell lines. Akt and p-Erk1/2 decreased significantly in most cases. Pan Akt, however, increased significantly at 72h in MCF7, and similarly in H1650 cells, as well as the p-Erk1/2 at 72h in MCF7 and at 10x dilution in A549. Phosphorylation of p53 (Thr55) decreased significantly in all cases, while activated p53 (Ser15) increased significantly at 72h in MCF7. Each bar represents the average normalized density from at least 3 repeats ± SD. Statistical significance are plotted as *: p < 0.033, **: p < 0.002, and ***: p < 0.001. C: control. 10x and 20x: concentrations of methyl-donors

3.2 Methyl-donor Treatments

L-methionine, choline chloride, folic acid and vitamin B12 were purchased from Sigma Aldrich (M5308, C7527, F8758, V6629, respectively; St. Louis, MO, USA). Cells were grown in culture media until 50% confluence then were treated with

different concentrations (1x, 10x, 20x) of the mixture of methyl-donors. Basal concentration (1x) was: 17 mg/L L-methionine, 9 mg/L choline chloride, 3 mg/L folic acid and 2 mg/L vitamin B12. These concentrations were used according to a previous study of Park et al. [36].

3.3 Cell Proliferation Assay

Cells were plated onto 96-well plate at a cell density of 1.5-3 x 10^4 cells/mL depending on cell lines. When cells reached 50% confluence, culture media were changed to methyl-donor supplemented media and incubated for 24-72 hours. Cell growth was measured at 24h, 48h or 72h by a colorimetric MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt;] cell proliferation assay (CellTiter 96® AQueous One Solution Cell Proliferation Assay, G3582; Promega, Madison, WI, USA) according to manufacturer's instructions. Briefly, 20µL of the reagent was added into 100µL of culture medium in each well at 24h, 48h or 72h of treatments. After 2 hours of incubation, the absorbance of the soluble formazan product was measured at 490 and 690 nm with a plate reader (Labsystems Multiscan MS, Thermo Fisher Scientific Waltham, MA, USA).

3.4 Detection of Apoptosis

Cells were plated onto 6-well plates at a cell density of 3-5 x 10^4 cells/mL depending on cell lines. After 24, 48, or 72 hours treatments of methyl-donor, cells were washed with PBS then detached by trypsin-EDTA solution (XC-T1717; Biosera, Nuaille, France). After centrifugation (1500 rpm, 5 minutes), apoptotic cell fraction was determined by using FITC Annexin V Apoptosis Detection Kit with PI (640914; BioLegend, San Diego, CA). Annexin V and/or PI positive cell fractions were detected by CytoFLEX flow cytometer using CytExpert software (Beckman Coulter, Indianapolis, IN, USA).

3.5 Cell Cycle Measurement

Tumor cell lines were plated and treated as described above in the section of detection of apoptosis. After washing steps, cells were fixed in ice cold 70% ethanol at room temperature for 20 minutes, then were kept at -20°C for additional 30 minutes. Cells then were washed twice in PBS. After resuspended in PBS containing 1% RNaseA (R5503; Sigma Aldrich, 10 mg/mL) 20 µL propidium iodide solution were added (P3566, Thermo Fischer Scientific Inc, Waltham, MA, USA; 1mg/mL) and the samples were incubated for 1 hour at 4°C. Cell cycles were detected by CytoFLEX flow cytometer using CytExpert software (Beckman Coulter, Indianapolis, IN, USA).

3.6 Western-blot

Cells were lysed in RIPA-buffer, supplemented with 0.5 mM Na-orthovanadate, 10 mM NaF and 1:200 Protease Inhibitor Cocktail (P8340, Sigma-Aldrich, St. Louis, MO, USA). Lysates were collected in tubes then centrifuged on 12,000

rpm for 15 minutes. Total protein concentration was determined using Pierce Rapid Gold BCA Protein Assay Kit (A53226; Thermo Fisher Scientific, Waltham, MA, USA). Cell extract were mixed with 5x sample loading buffer containing 2-mercaptoethanol (1610710; Bio-Rad, Hercules, CA) and heated to 95°C for 5 minutes.

Table 1. Specifications and dilutions of the applied primary and secondary antibodies

Name	Manufacturer	Cat. number	Dilution	Host
Caspase-9	Cell Signaling	9502S	1:1000	rabbit
Bak (D4E4)	Cell Signaling	12105T	1:1000	rabbit
Puma	Cell Signaling	12450T	1:1000	rabbit
Bax (D2E11)	Cell Signaling	5023T	1:1000	rabbit
Bcl-2 (124)	Cell Signaling	15071S	1:1000	mouse
Mcl-1 (D5V5L)	Cell Signaling	39224S	1:1000	rabbit
p21 (SX118)	Santa Cruz Biotechnology	sc-53870	1:200	mouse
p-Erk1/2	Cell Signaling	4370S	1:2000	rabbit
p-p53 (B-3) (Thr55)	Santa Cruz Biotechnology	sc-377553	1:200	mouse
p-p53 (Ser15)	Cell Signaling	9284T	1:1000	rabbit
p-Akt	Cell Signaling	3787S	1:1000	rabbit
Akt (pan) (11E7)	Cell Signaling	4685S	1:1000	rabbit
beta-Actin (13E5)	Cell Signaling	4970S	1:5000	rabbit
Anti-mouse IgG, HRP-linked	Cell Signaling	7076S	1:1000	horse
Anti-rabbit IgG, HRP-linked	Cell Signaling	7074S	1:1000	goat

For Western blot analysis, 12-30 ug of total proteins were loaded and run in 10% sodium dodecyl sulphate polyacrylamide gel (SDS-PAGE) at 80V for 20 minutes than at 180V for 50 minutes on Mini Protean vertical electrophoresis equipment (Bio-Rad, Hercules, CA). Proteins were transferred onto Immobilon-P PVDF transfer membrane (IPVH00005; Merck KGaA, Darmstadt, Germany) by blotting at 100V for 60 minutes at +4 °C. Membranes were blocked, then incubated with primary antibodies overnight at +4°C (dilution in Table 1). β-actin was used for loading control. After washing steps, membranes were incubated with HRP-labelled secondary antibodies (Table 1) for 60 minutes at room temperature. Membranes were detected using SuperSignal West Pico Chemiluminescent Substrate Kit (34080; Thermo Fisher Scientific, Waltham, MA, USA) and were visualized by iBright FL1500 Imaging System (Thermo Fisher Scientific, Waltham, MA, USA). Densitometric analysis of the immunoblots was performed using Image J software (developed by NIH and LOCI, University of Wisconsin).

3.7 Statistical analysis

All experiments were repeated at least n = 3 different times and are expressed as mean ± standard deviation (mean ± SD). We applied the non-parametrical t-test Wilcoxon matched-pairs signed rank test, one-way ANOVA and two-way ANOVA with Bonferroni or Geisser-Greenhous correction, where Dunnett test was used for multiple comparisons. We applied the GraphPad Prism software (GraphPad Software LLC, San Diego, CA, USA). Significance level was used as *: $0.01 < p < 0.05$, **: $0.001 < p < 0.01$, and ***: $p < 0.001$ in case of proliferation, cell cycle experiments and measurement of the level of apoptosis. Analyzing Western blots, the p value style of NEJM was used (*: $0.002 < p < 0.033$, **: $0.001 < p < 0.002$, and ***: $p < 0.001$).

4. DISCUSSION AND CONCLUSION

Several earlier studies have suggested that dietary methyl-donors could contribute to cancer prevention as high intake of methyl-donors and related vitamins could reduce the risk of breast and lung cancers [10,11,12]. Accumulating data have shown recently how dietary vitamins and herbal extract can influence signaling pathways both in normal and tumor cells [36-39]. In this work, we further studied the effects of methyl-donors (using a mixture of L-methionine, choline chloride, folic acid and vitamin B12) on breast (MCF7 and T47D) and lung (A549 and H1650) cancer cell lines. We could confirm our starting hypothesis by showing that methyl-donor treatment can reduce tumor cell proliferation rate and activate cell death related pathways resulting in measurable programmed death response of cancer cells. Since methyl-donor treatment affected the proliferation rate in all tested cell lines, we focused on two main growth and proliferation pathways MAPK/ERK and PI3K/AKT, which are most frequently involved in tumorigenesis [39]. Indeed, p-Erk1/2 protein levels were significantly decreased after the treatments. The MAPK/ERK pathway is known to phosphorylate and thus inactivate Caspase-9 and subsequently inhibit apoptosis [40]. This was further supported by the post-treatment downregulation of Akt levels. The PI3K/AKT pathway is also frequently upregulated in breast cancers and associated with metastatic growth, chemotherapy resistance and poor prognosis [41]. *AKT1* overexpression has pro-proliferative and anti-apoptotic effect [42,43], and its knockdown can revert these activities [44]. Hypoxia induced *AKT2* can induce cancer proliferation [45] while downregulated *AKT2* is linked with cell cycle arrest and reduced proliferation [46,47]. Of the 4 cell lines only at 72h of MCF7 breast and H1650 lung cancer showed elevated Akt levels upon methyl-donor treatment, which, however, was not associated with elevated proliferation, at the latter probably due to the compensation by the reduced p-Erk1/2 levels.

These were in line with the findings by another group showing significantly reduced proliferation in both MCF7 and T47D cells after methyl-donor treatment besides detected significantly decreased expression of Bcl-2 protein in T47D, but not the MCF7 cell lines [36].

Thus, we analyzed the apoptotic process, and additionally, the cell cycle changes, focusing on the SubG1 fractions, which contains the apoptotic cells as well. Although we detected significantly increased subG1 fraction only in breast cancer cell lines, a similar tendency was seen also in the NSCLC cells. The Annexin-V positive cell fractions after treatments revealed that the number of the single positive, early apoptotic cells increased significantly in T47D, A549 and H1650 cell lines, except in MCF7 cells.

In line with the elevated apoptosis we tested the expression of the pro- and anti-apoptotic pathway proteins after methyl-donor treatment. We confirmed the increased expression of the pro-apoptotic proteins Bak and Bax, which are involved in mitochondrial membrane permeabilization [48]. This was in line with the significantly elevated level of Caspase-9 detected in cases (except T47D), which is activated in the apoptosomes upon mitochondrial membrane damage by the potentially released cytochrome C [40,48]. As a further support of apoptosis pathway activation, we found reduced anti-apoptotic Mcl-1 protein levels, which could have prevented mitochondrial membrane damage against Bak and Bax activity [49-51]. Although, none of these changes were significant in T47D cells, Annexin-V indicated apoptosis and elevated subG1 fraction was detected also in these cells. Surprisingly, in MCF7 cells the anti-apoptotic Bcl-2 levels increased after treatment, which (along with Bcl-xL) can inhibit Puma to mediate apoptosis resistance [52]. Indeed, we detected significant decrease of Puma levels and lack of treatment induced an increase of early apoptotic cells in MCF7. As opposed to Mcl-1, we found the dose dependent significant reduction of Bcl-2 only in H1650 cells after the treatment.

Phosphorylation of p53 at Ser15 and Thr55 play important role in the nucleocytoplasmic shuttling of p53 [53]. Inhibition of p-p53 (Thr55) restores the nuclear localization of p53 and sensitizes to DNA-damage [54,55], while DNA-damage induces phosphorylation at Ser15 [56,57]. Therefore, the reduced p-p53 (Thr55) levels after methyl-donor treatment may restore functioning p53 and allow subsequent activation of downstream pro-apoptotic signaling. Curcumin and vitamin E can downregulate p-p53 at Ser15 to protect against cytotoxicity caused by chemotherapeutic agents in normal lung epithelial cells [58]. We expected from nutrients to support normal cell functions and sensitize the tumor cells to subsequent targeted therapies, therefore not expected increased phosphorylation of p53 (Ser15) without chemotherapies or DNA-damaging agents. We can conclude that, methyl-donor treatment may restore p53 nuclear localization and protect against unnecessary metabolic stress, while induce apoptosis in cancer cells.

Vitamin C induce apoptosis, without affecting p53 [59] and mediates anti-proliferative effects as well in several drug resistant breast cancer cell lines [60]. Additionally, has synergistic effect with chemotherapies [61], without having a significant impact on normal cells [59]. Similarly, vitamin B2 sensitize cancer cells to vitamin C induced cell death [62]. Although, vitamin B1 (thiamine) did not induce apoptosis, but reduced cell viability selectively on cancer cells, with significant increase of basal, maximum, and ATP production oxygen

consumption in MCF7 cell, but not in MCF10A. Moreover, reduced the extracellular lactate levels of both cancer and normal cell lines, and increased cellular pyruvate dehydrogenase (PDH) activity in breast cancer MCF7 cells [38]. When PDH activity is decreased, glycolysis replaces the failed aerobic metabolism, which results in elevated lactate level [63,64]. Tumor cells prefer glycolysis for tumor growth, thus restoring the aerobic metabolism slows down the tumor growth [65]. Methyl-donors have only moderate, non-significant effect on normal cell growth, similarly to vitamin C [36,59].

Appropriate diet, rich in complex food intake, including ω-3 fatty acids, fruits and vegetables can be used to counteract cancer-related fatigue (CRF), particularly in patients with breast cancers [66]. Although, the lack of standardization in dietary interventions and heterogeneity of study design, nutrition therapies and quality of life measures may not allow to draw firm conclusions, preliminary data indicate that plant-based nutrition therapy can support to CRF [67,68].

In conclusion, dietary methyl-donors may contribute to reducing MAPK/ERK and AKT pathways and protecting p53 functions, which may sensitize tumor cells to chemotherapy induced DNA-damage. Methyl-donors are also supposed to reduce cytotoxicity in normal cells and unnecessary metabolic stress in cancer cells to buffer the adverse effects of oncotherapies. Further studies are required to confirm the effectiveness methyl-donors and to standardize their application in the clinical setup.

AUTHOR CONTRIBUTIONS

This work was carried out in collaboration among all authors. Author EK did the execute experiment, data analysis, paper writing, paper review. Author GF did the paper review. Author RM did the paper review. Author MD did the funding TK: resources, paper review. Author IT did the resources, paper review. Author ZN did the conception, design, execute experiments, data analysis, paper writing and final approval. All authors read and approved the final manuscript.

FUNDING

This research was funded by Ministry of Hungarian National Technology and Innovation (NVKP_16-1-2016-0042) and by the ÚNKP-21-4-I-SE-19 New National Excellence Program of the Ministry for Innovation andTechnology from the source of the National Research, Development and Innovation Fund obtained for Eva Kiss.

ACKNOWLEDGMENTS

We would like to thank to Éva Mátrainé Balogh for her technical assistance (1st Department of Pathology and Experimental Cancer Research, Semmelweis University, Budapest, Hungary).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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This chapter is an extended version of the article published by the same author(s) in the following journal.
International Journal of Molecular Sciences, 22, 3598, 2021.

Chapter 6
Print ISBN: 978-93-5547-742-2, eBook ISBN: 978-93-5547-749-1

Pediatric Pancreatitis: Demography, Etiology, Complications and Ultrasound Findings in a Tertiary Care Centre

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DOI: 10.9734/bpi/cpms/v9/16987D

ABSTRACT

Pancreatitis is less common in children and adolescents than in adults, and the causes in adults and children are different. The objective was to study demography, etiology, complications and detail ultrasound findings in pediatric pancreatitis. Retrospectively, 20 patients were evaluated for demographics, etiology, complications, and transabdominal ultrasound findings showing a common presentation in the 10- to 15-year-old age group with predominantly female patients. Acute and chronic pancreatitis were noted in two and eighteen cases, respectively. Idiopathic cause and chronic variant most commonly observed in our study population. Pseudocyst and ascites were the most common complications of pancreatitis found in our study. Ultrasound is an important first-line test that can be used in the diagnosis, classification, and follow-up of pediatric pancreatitis.

Keywords: Ultrasound; pancreatitis; paediatric.

1. INTRODUCTION

The pediatric onset of pancreatitis is indicated when the first episode of acute pancreatitis occurs before the patient's 19th birthday [1]. Pancreatitis in children is less common than pancreatitis in adults. But it is associated with significant morbidity. Although the disease is well characterized in adults, there is limited data in the pediatric population. Image data is also limited. Transabdominal ultrasound is an inexpensive, readily available first-line test that can be used in the diagnosis, classification, and follow-up of pediatric pancreatitis. Computed tomography (CT) and magnetic resonance imaging (MRI) can be used to assess severity, but CT scans carry risks of radiation hazards, and MRI is expensive, time-consuming, and requires sedation [2]. In such a scenario, transabdominal

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ultrasound is very helpful for the periodic follow-up of pediatric patients. Through endoscopic ultrasound is widely used in the adult population, research is ongoing into its safety and feasibility in the pediatric population [3]. Here we share our experience regarding the demographics, etiology, complications, and transabdominal ultrasound findings of pediatric pancreatitis in a tertiary care hospital.

2. MATERIALS AND METHODS

Patients from gastroenterological department of our hospital were retrospectively examined in the period from January 2012 to August 2014. Clinical information, complications, and laboratory data were collected through a standardized review of medical records and the data recorded in data forms. The ultrasound image data was collected from our departmental archived collection. The diagnosis of pancreatitis was confirmed on the basis of clinical, radiological and laboratory findings. Diagnosed cases of pancreatitis with onset of symptoms under 18 years of age were included in our study. Sonographic evaluation was performed on a Philips HD 11XE equipped with a 3.5 to 5 MHz curve transducer and a 7 MHz linear array transducer. The patients were examined after a 4-hour fasting period and in the supine position. Patients with successful imaging of the pancreas and evidence of the pancreatic duct were included in the study.

Imaging was epigastric, transhepatic, and transplenic. In the epigastric approach, imaging was performed in transverse and oblique planes. The transhepatic approach was used for the head of the pancreas and the transplenic approach for the tail of the pancreas. Sometimes water was given to demonstrate the pancreas by creating an optimal window for better ultrasound transmission. Acute pancratitis was diagnosed by clinical laboratory findings and evidence of a voluminous, hypoechoic pancreas. Chronic pancreatitis was diagnosed on the basis of clinical features (abdominal pain, diabetes mellitus) and evidence of pancreatic duct and/or parenchymal changes (calcification, atrophy, duct dilatation) in imaging. Morphologic changes in the pancreas were identified by abdominal ultrasound. The main pancreatic duct was considered dilated if the duct diameter was greater than 3 mm in the head and 2 mm in the body or tail of the pancreas.

3. RESULTS

Of a total of 20 patients, five were male and fifteen were female. Age distribution revealed 1 in the age group 0-5 years, 5 in 5-10 years, 8 in 10-15 years, 6 in over 15 years (Fig. 1). A maximum of eight patients were in the 10-15 year age group. Regarding the etiology, we found 15 idiopathic cases, 2 traumatic cases, 1 congenital case, 1 case of infection and 1 case of biliary disease. Pain was the main complaint in all of these patients. Two cases were of acute and eighteen cases of chronic pancreatitis.

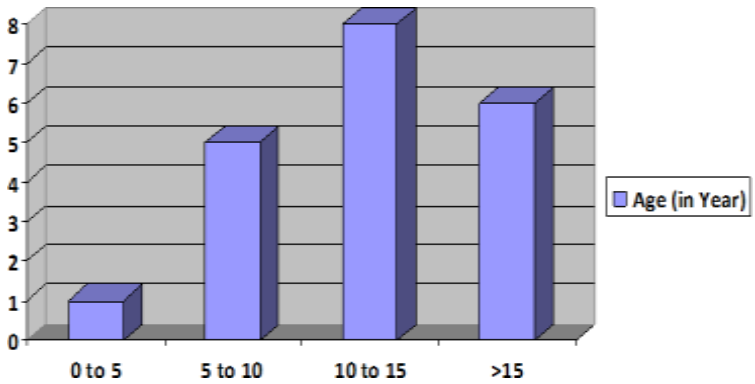

Fig. 1. Chart diagram showing age distribution of our patients

Morphological changes of the voluminous and hypoechoic pancreas with peripancreatic edema were found in both cases of acute pancreatitis (Fig. 2). Single or multiple changes of chronic pancreatitis were observed (Fig. 3). Chronic pancreatitis changes of pancreatic calcification, duct dilatation, parenchymal atrophy found in eight cases. Duct dilatation and parenchymal atrophy were found in five cases. Pancreatic calcification, duct dilatation was observed in two cases. In one case pancreatic calcification and parenchymal atrophy were found. Pancreatic calcification in one case and parenchymal atrophy in another case. Idiopathic calcifying pancreatitis was found in ten cases and idiopathic non-calcifying pancreatitis was observed in the remaining cases. Idiopathic chronic calcified pancreatitis was observed in four out of five men.

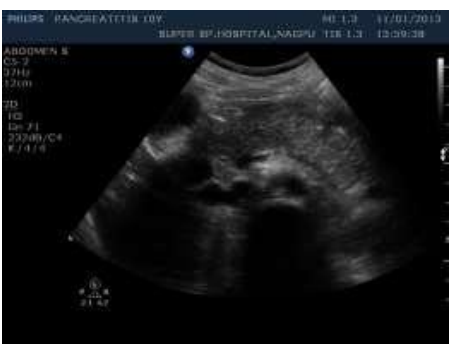

Fig. 2. Ultrasound image of pancreas in acute pancreatitis showing bulky, hypoechoic pancreas

Diabetes mellitus was observed in two cases of idiopathic calcific pancreatitis. Pseudocysts (Fig. 4A) were present in four cases, ascites was present in four, and multiple effusion was present in one case. In the remaining cases, no complications were observed.

Table 1. Morphological changes of chronic pancreatitis in our patients

Sl. No.	Morphological changes in Chronic pancreatits.	Cases
1	Pancreatic calcification, duct dilatation and parenchyma atrophy	8
2	Pancreatic calcification, duct dilatation	2
3	Duct dilatation and parenchymal atrophy	5
4	Pancreatic calcification and parenchymal atrophy	1
5	Pancreatic calcification	1
6	Duct dilatation	1

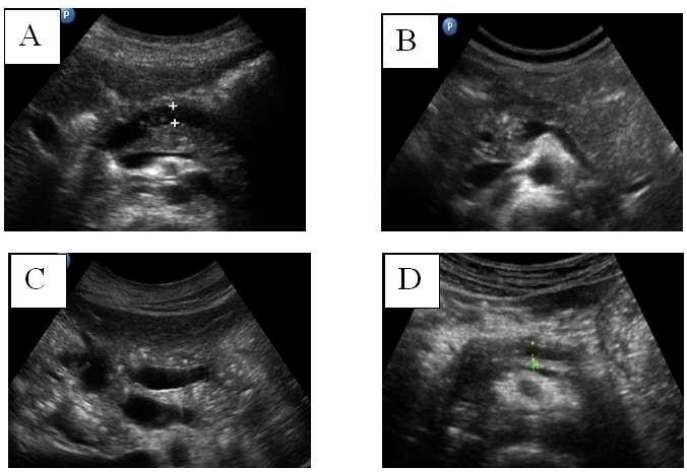

Fig. 3. Ultrasound images of pancreas in chronic pancreatitis showing pancreatic duct dilatation in case A, pancreatic calcifications in head of pancreas in case B, pancreatic duct dilatation with pancreatic calcification in case C pancreatic duct dilatation with parenchymal atropy in case D

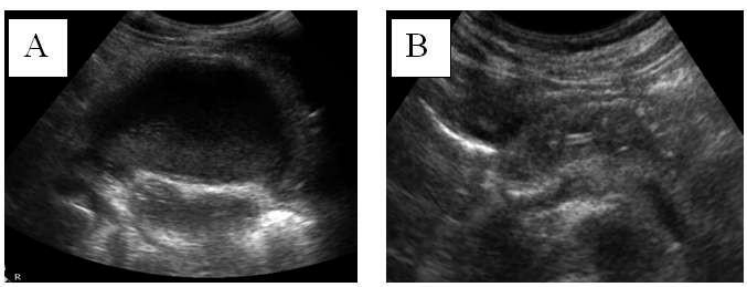

Fig. 4. A) Ultrasound image showing pseudocyst of pancreas in retroperitoneal region. B) Ultrasound image showing pancreatic stent in main pancreatic duct in a case of chronic pancreatitis

4. DISCUSSION

According to INSPIRE (International Study Group on Pediatric Pancreatitis In Search of a Cure), the diagnosis of acute pancreatitis requires at least two of the following three criteria: a) abdominal pain with acute onset, particularly in the epigastric region, b) serum amylase and/or lipase activity of at least 3 times greater than the upper limit of normal, c) Imaging findings consistent with acute pancreatitis [2,4]. Acute pancreatitis is an inflammatory disease characterized by the release of pancreatic enzymes from the pancreatic duct into the pancreatic and retroperitoneal regions [4]. Acute recurrent pancreatitis requires at least 2 distinct episodes of acute pancreatitis as defined above along with complete pain relief and 1 month of pain free interval between diagnoses of acute pancreatitis or a complete normalization of serum amylase and lipase before the next episode of acute pancreatitis together with a complete disappearance of pain symptoms, regardless of a certain time interval between episodes of acute pancreatitis [4].

Chronic pancreatitis is a chronic progressive inflammatory disease that causes irreversible damage to the pancreas, resulting in exocrine and endocrine insufficiency. Abdominal pain is a predominant symptom of chronic pancreatitis, which can lead to a significant impairment of quality of life [5]. Steatorrhea and diabetes mellitus are the long-term consequences of chronic pancreatitis [6,7]. Chronic pancreatitis requires at least one of the following three criteria: a) abdominal pain suggestive of pancreatic origin and imaging findings of chronic pancreatic injury, b) evidence of exocrine pancreatic insufficiency along with imaging findings of chronic pancreatitis, c) evidence of endocrine pancreatic insufficiency together with pancreatic imaging findings of chronic pancreatitis [4].

Recent studies reported the increasing incidence of pediatric acute pancreatitis between 3.6 and 13 cases per 100,000 pediatric population per year. The median age of 9.2 ± 2.4 (SD) years and a male to female ratio of 1:2 were reported in a meta-analysis of acute pancreatitis in children and adolescents [2].

There are several causes that can lead to pancreatitis. In pediatric patients, abdominal trauma, pancreatic duct abnormalities, viral infection, familial idiopathic pancreatitis, autoimmune process, drug therapy, hyperlipidemia, biliary disease, idiopathic infection are reported causes. Trauma is one of the main causes of pancreatitis in children [2,8]. Pancreatic injury occurs in 10% of abdominal trauma [9]. The body of the pancreas is located anterior to the vertebral body and is generally predisposed to injury in abdominal trauma. Child abuse, bicycle-related injuries, sports injuries, car accidents, and penetrating trauma are causes of traumatic pancreatitis. Alcohol abuse and gallstone disease are the most common causes of pancreatitis reported in the adult population. However, these causes are rare in the pediatric population [8].

Structural abnormalities such as pancreatic divisum and cholochal cyst can cause pediatric pancreatitis. Choledochal cyst occasionally presents as pancreatitis. Pancreatic divisum requires a concomitant disease such as papillary

stenosis to induce pancreatitis [8]. Hereditary pancreatitis is a common cause of recurrent pancreatitis in children. Diagnosis of hereditary pancreatitis requires evidence of pancreatitis in three or more family members and a history of recurrent abdominal pain since childhood with no etiological cause [10]. Autoimmune pancreatitis is one of the components of the IgG4 systemic disease, which is a chronic inflammatory process affecting multiple organs. It is rare and till 2011 about 9 cases have been reported in the literature. Three imaging patterns: diffuse, focal, multifocal have been reported for autoimmune pancreatitis [2]. Azathioprim, sulfonamides, hydrocholorothiazides, L-asparaginase, vincristine, and corticosteroids have been reported to cause drug-induced pancreatitis. Treatment of acute lymphoblastic leukemia (ALL) can lead to chemotherapy-induced pancreatitis [11]. Hyperthyroidism, hyperlipidemia, cystic fibrosis are metabolic causes that need to be ruled out and structural causes that need to be ruled out in ERCP [8]. Systemic lupus erythematous, chronic renal failure, sepsis, inflammatory bowel disease, cystic/fibrosis, diabetic mellitus are systemic diseases that cause or are associated with pediatric pancreatitis [2]. The most common cause in our study was idiopathic. Previous studies had indicated a range of 6 to 33% [12, 13].

Abdominal pain, nausea, and vomiting are the most common complaints reported in pediatric pancreatitis. Abdominal pain is localized diffusely or epigastrically. Assessing abdominal pain is a very difficult task. Abdominal pain was the most common symptom found in our study. Nausea and vomiting have also been reported in some patients. Serum amylase is elevated in up to 95% of patients with pancreatitis. In our patients, it was found that amylase was elevated in all patients. It can be normal in pancreatitis and increased after 12 hours in acute cases. In most cases, LDH, TLC, and total bilirubin levels can be normal or slightly elevated [8].

Abdominal ultrasound has demonstrated an 80% accuracy in assessing pancreatitis. Ultrasound imaging visualization of the pancreas was reported in 75-93% of cases. In a mild and early stage of acute pancreatitis, transabdominal ultrasound may be normal or equivocal [2]. A limited sensitivity of 47% was reported by Richardson et al. in the detection of chemotherapy-induced pancreatitis in ALL patients [11]. It is also useful to rule out other causes of abdominal pain, such as: acute appendicitis, renal colic, intestinal obstruction, etc. Acute pancreatitis usually shows increased pancreatic volume and decreased pancreatic echogenicity [5]. A recent study by Trout et al. showed that peripancreatic edema (54-63%) was common in pediatric acute pancreatitis compared to duct dilatation (12-14%) [14]. We found peripancreatic edema in all two cases of acute pancreatitis without ductal dilatation. Pancreatic atrophy, calcifications, duct dilatation are observed in chronic pancreatitis [15]. Ultrasonography is a noninvasive, safe, widely used, and inexpensive imaging modality of first choice in the evaluation of pancreatitis. It is also important in tracking known cases of pancreatitis and detecting the various complications early on. The main disadvantage of ultrasound is operator dependency, and intestinal gases can obscure visualization of the pancreas. Ultrasound color

Doppler is important to detect vascular complications such as arterial pseudoanurysm and splenic vein thrombosis.

Endoscopic ultrasound (EUS) is a minimally invasive imaging tool designed to better visualize pancreatic and biliary disease compared to transabdominal ultrasound. EUS-guided fine-needle aspiration (EUS-FNA) of a solid pancreatic, biliary lesion and therapeutic aspiration of a cyst can be performed in the same setting during EUS. Altonbary AY et al. had reported that EUS and EUS-FNA are safe and feasible, with significant clinical implications for management in the pediatric population [3]. Ultrasound elastography can detect hard areas in solid lesions that can be targeted to EUS-FNA for the best diagnostic tissue sampling [3]. Computed tomography (CT) is useful for suspected pancreatitis, in which the pancreas is obscured by intestinal gases on ultrasound and to assess severity by CT severity index. In cases of traumatic pancreatitis, CT can also examine the liver, spleen, gastrointestinal tract, spine, or other organs that may be involved in abdominal trauma [8]. MRI has a role in assessing the severity of pancreatitis without radiation risk. More sensitive than ultrasound in detecting choledocholithiasis, MRCP is a non-invasive imaging tool used to visualize ductal anatomy and abnormalities [2].

Endoscopic retrograde cholangiopancreatography (ERCP) is a valuable investigation in the diagnosis and management of chronic pancreatitis in children. 2% morbidity is reported in children, mainly with mild pancreatitis [16,17]. Relative contraindications for ERCP are acute pancreatitis and a pancreatic pseudocyst that is not staged for surgical drainage. ERCP should be considered in the evaluation of idiopathic nonresolving or recurrent pancreatitis and in all children with pseudocysts prior to surgery. Diagnosis of pancreatitis is generally based on clinical, laboratory, and imaging findings [8]. Pseudocyst formation is the most common complication, reported in 10-25% of childhood pancreatitis cases. Most of them are acute and thin-walled. Biliary obstruction as a result of acute or chronic pancreatitis is rare in children and adolescents [18,19]. Other complications are pseudoanurysm, venous thrombosis, pancreatic-pleural fistula [2]. In our patients, pseudocyst and ascites were the most common complications, and biliary obstruction was not reported.

Bowel rest and IV fluids with or without nasogastric suction are the first line of treatment, and conservative management occurs in 30-76% of cases [12,13,20]. Pancreatic pesudocyst drainage is required in some cases and gives better results compared to adults because there is no primary pancreatic lesion. Pseudocyst drainage was performed in two cases. Cholecystectomy with intraoperative cholangiogram or CBD exploration is also performed for gallstone pancreatitis. Other surgical treatments such as spincterotomy, spincteroplasty, stenting, biliary bypass surgery (choledochoduodenostomy, choledochojejunostomy) have also been reported [6]. Chaudhury et al. had reported on two subgroups of idiopathic chronic pancreatitis, such as calcific idiopathic chronic pancreatitis and non-calcific idiopathic chronic pancreatitis. Calcific idiopathic chronic pancreatitis was characterized by male predominance, early calcification, and a high frequency of endocrine insufficiency. Noncalcific idiopathic chronic pancreatitis had shown a same-sex distribution, no

calcification, and a low incidence of endocrine insufficiency [21]. Our study also demonstrated male predominance and endocrine insufficiency in calcific idiopathic chronic pancreatitis.

Exocrine pancreatic insufficiency can result from pancreatitis. Diabetes is reported in 2 cases. Long-term abdominal pain also occurs with pancreatitis, which was observed in 7 cases. The average length of hospital stay for our patients was 6 days. According to the literature, the median length of stay in hospital in 2009 was 4 days [2]. The most common long-term complication reported was recurrent pancreatitis, which was observed in 7 cases. Almost 25% of acute pediatric pancreatitis develop serious complications [2]. The mortality in pancreatitis varies between 0 and 78% [13]. In our study we found that it is 0%. The overall mortality is about 18-23%. The Atlanta classification divided acute pancreatitis into mild and severe forms. In a mild form, it is very low (1%) in both children and adults. In severe form, mortality is higher in the pediatric adolescent age group (30-35%) than in the adult group (20-25%) [1].

5. CONCLUSION

Pancreatitis is an uncommon disease in children but can result in significant morbidity. In our study, pancreatitis was more common in 10-15 years age group with female predominance and pseudocyst was the most common complication. Enlarged pancreas with peripancreatic edema observed in acute pancreatitis. Idiopathic chronic pancreatitis was the predominant form of chronic pancreatitis found in our study. It is important to distinguish between calcified and non-calcified variety as the calcified variety is characterized by early morphological and functional damage. This differentiation can be done on ultrasound. Because of its accuracy, noninvasiveness, speed, portability, and relative inexpensiveness, any suspected case of pancreatitis in children and adolescents should still be investigated and followed with transabdominal ultrasound.

ACKNOWLEDGEMENT

Special thanks to SJAMS journal from SAS publishers to permit reuse of article content, table and images from “Ultrasound Assessment of Pancreatitis in Paediatric Adolescent Population”.

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This chapter is an extended version of the article published by the same author(s) in the following journal.
Scholars Journal of Applied Medical Sciences (SJAMS), 2(6D):3140-3144, 2014.

Chapter 7
Print ISBN: 978-93-5547-742-2, eBook ISBN: 978-93-5547-749-1

Meniscal Injuries: Review

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DOI: 10.9734/bpi/cpms/v9/3384A

ABSTRACT

Meniscal injuries are common cause of pain and functional impairment in knee. Proper clinical examination and evaluation is must to diagnose the injury. Treatment in form of physical therapy can help sone patients, while surgical options like meniscal repair, partial menisectomy or total menisectomy is needed in some patients. Proper rehabilitation after surgery is required to get good functional results

Keywords: Meniscal injury; menisectomy; meniscal repair; knee pain.

1. INTRODUCTION

Meniscal injuries are a common cause for pain and functional impairment of the knee joint. Initially it was thought that meniscus was a unnecessary appendage and could be sacrificed [1]. It was only after Fairbank described radiographic changes in the knees following menisectomy and poor results of total meniscectomy that conservative approach towards meniscal injuries was considered [2]. This meniscal preservation lately has led to development of new surgical techniques to restore the native structure of the meniscus so as to restore function and biomechanics of the knee.

2. ANATOMY

On gross examination menisci are smooth, lubricated tissue. They are crescent-shaped wedges of fibrocartilage located on the medial and lateral aspects of the knee joint. The wedges are flat on the tibial side and concave on the femoral side to accommodate the femoral articular surface. The peripheral one third of the wedge is thick and vascular often called as Red zone, the wedge tappers on inner border and is avascular in inner two thirds, also referred to as white zone (Fig. 1). [3]. Both menisci differ from one another in shape.

Medial meniscus: The medial meniscus is C shaped and occupies about 50% of the area of the medial compartment. The anterior horn of medial meniscus is attached firmly to the tibia anterior to the anterior cruciate ligament (ACL). The

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posterior horn is attached in front of the attachment of the posterior cruciate ligament (PCL). The outer border of the medial meniscus merges with the knee joint capsule making it less mobile and prone to injuries. The coronary ligament attaches the meniscus to the upper tibia.

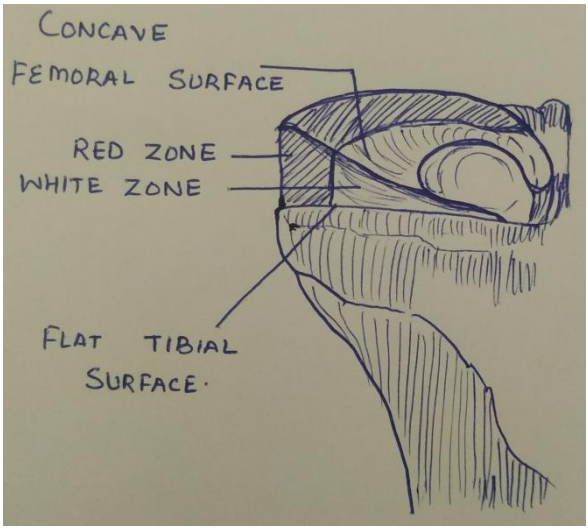

Fig. 1.

Lateral meniscus: The lateral meniscus covers 70% of the lateral tibial plateau. The transverse (intermeniscal) ligament attaches the anterior horns of the lateral and medial menisci. The posterior horn of the lateral meniscus is attached to the PCL and medial femoral condyle through the meniscofemoral ligaments of Wrisberg (the posterior meniscalfemoral ligament) and Humphrey (the anterior meniscal-femoral ligament). It is also attached to the popliteus tendon [4]. The lateral meniscus is more mobile and is not anchored to the lateral collateral ligament. The anchor of the lateral meniscus to the femur and the popliteal tendon couples its motion with that of the femoral condyle during rotation. It is therefore less likely to be injured. Discoid lateral meniscus is a variant of lateral meniscus. It was thought to be due to developmental arrest, however Clark and Ogden proved otherwise [5].

The main function of the menisci is tibiofemoral load transmission, shock absorption, and lubrication of the joint [6]. The menisci compensate for significant incongruity between the femoral and tibial articulating surfaces. The human menisci transmit 30–55% of the load in a standing position [6]. After meniscectomy, tibiofemoral contact area decreases leading to contact stresses these changes ultimately cause joint degeneration. They also help to distribute synovial fluid throughout the joint and aid in the nutrition of the articular cartilage.

The menisci also contribute to the stability of the knee, largely as secondary soft tissue restraints which prevent anterior tibial displacement.

3. CLINICAL PRESENTATION

The meniscal injuries have a bimodal age distribution in young active sports person and in elderly people. In young people most common reason is a non contact sports injury which occurs while decelerating, rotating on knee, or landing and jumping on the knee. However in old age tear is mostly degenerative with patient unaware of it until mechanical symptoms occur. Locking, buckling, catching are suggestive symptoms of a meniscal injury. Some patients may even claim to have heard popping sensations during injury. Swelling often occurs a day or two later, immediate swelling usually is a sign of bleeding into the knee. The swelling associate with the meniscal injury may be recurrent.

Clinical evaluation starts with the gait examination, a painful limp is usually present more so in acute injuries. Care should be taken to access thigh muscle wasting, knee joint effusion and joint line tenderness. Proper examination for ligamentous and other soft tissue injuries should be done to rule out injury to these structures. The stability of the knee should be assessed for concurrent ligamentous injury.

Following specific tests for meniscal injuries have been described.

McMurray test: with patient supine, hip and knee is flexed, medial meniscus is accessed by external rotation of foot and extension of knee, while lateral meniscus is accessed by internal rotation of foot and extension of knee. Feeling of a clunk is considered a positive test. A specificity of 100% is reported with this test [7].

Apley's Grind test: This test is used to distinguish between meniscal and ligamentous involvement. With the patient in a prone position, the knee flexed at 90°, and the leg stabilized by the examiner's knee, distract the knee while rotating the tibia internally and externally. Pain during this maneuver indicates ligamentous involvement. Then, compress the knee while internally and externally rotating the tibia again. Pain during this maneuver indicates a meniscal tear. This test is currently discouraged [7].

Bounce home test: The patient is supine with heel held in the examiner's hand. The examiner fully flexes the knee and then passively extends the knee. If the knee does not reach complete extension or has a rubbery end feel, test is considered positive. The knee movement may be blocked by a torn meniscus.

O'Donoghue test: With the patient prone, the examiner flexes the knee 90°. The examiner rotates the tibia internally and externally twice, then fully extends the knee and repeats the rotations. Increased pain during rotation in either or both knee positions indicates a meniscal tear or joint capsule irritation. With a valgus force to a flexed and laterally rotated knee, the medial meniscus, medial

collateral ligament (MCL), and the ACL all may be injured, representing the O'Donoghue triad.

Thesally test: The examiner supports the patient by holding his or her outstretched hands while the patient stands flat footed on the floor. The patient then rotates knee and body, internally and externally, three times, keeping the knee in slight flexion 5°. The same procedure is then carried out with the knee flexed at 20°. The test is always performed first on the normal knee first. Discomfort or sense of catching or locking is considered positive [8].

The reliability of the different tests and signs for meniscal lesion has been studied by several authors.

Fowler et al evaluated the predictive value of common clinical tests for the diagnosis of meniscal tears in 161 patients. They evaluated joint line tenderness, pain on forced flexion, the presence of a positive McMurray test, positive Apley grind, and distraction tests, and the presence of a block to extension. They compared clinical findings with arthroscopic findings. The authors have found that no one test was predictive for the diagnosis of a meniscal tear [9].

4. RADIOLOGICAL EVALUATION

Plain Xrays of the knee should always be asked. These help to rule out any bony injury, arthritic changes, loose body and joint malalignment.

MRI is the benchmark for the non invasive investigation for the meniscal injuries. Meniscal signals shown by MRI have four grades:

- Uniformly low signal intensity (normal meniscus) : Grade 0
- Irregular increases in intrameniscal signal: Grade 1
- Linear increased signal patterns not extending to meniscal surface: Grade 2
- Abnormal signal extends to the articular surface: Grade 3

While grades 0–2 have no surgical significance, grade 3 represents a meniscal tear [10,11]. One of the reasons for many false positive MRI reports is over interpretation of grade 2 signals.

Several studies have shown equal accuracy for clinical examination and MRI in diagnosing meniscal tear, however MRI helps to access the extent, location and type of tear, besides any associated cruciate ligament or chondral injury [12-14].

5. CLASSIFICATION

Meniscal tears have been classified in various ways depending on aetiology, location, pattern and MRI findings. However the most reliable and valid classification is the International Society of Arthroscopy, Knee Surgery and

Orthopaedic Sports Medicine classification [15]. Which is a elaborate classification taking into account Tear depth, Rim Width, Radial Location, Tear pattern, Quality of the tissue, Length of tear percentage of meniscus (surface area) that was excised.

6. TREATMENT

With the reports of osteoarthritis in knees after menisectomy and keeping in view the functions of the meniscus, preservation, repair or reconstruction of meniscus is now the standard form of care. The choice of treatment usually should take into account age, activity level, aetiology, patients expectations and lesion morphology.

Conservative treatment: is often considered for stable small peripheral vertical tears. Conservative treatment is also first line treatment in degenerative tears in elderly unless locking is present. Non operative treatment is also the first treatment in acute knee trauma in form of protection, rest, ice, compressions and elevation PRICE regimen. A conservative trail of at least 3 to 6 months is warranted if mechanical symptoms don't exist [16]. A recent RCT showed that patients who underwent surgical debridement and physiotherapy showed equally good results as patients who received physiotherapy only [17].

Surgical procedure might be necessary if there is locking or if symptoms persist for more than 6 months after injury.

Surgical treatment: Diagnostic arthoscopy is often necessary to determine the optimal treatment for the meniscal lesion. The main surgical procedures for the meniscal lesions can be braoadly divided into menisectomy, meniscal repair and meniscal reconstruction.

Menisectomy: Due to evidence of knee joint degeneration and altered bio mechanics of knee joint after total menisectomy, total menisectomy is rarely done these days. Partial menisectomy is usually done in radial tears in avascular zone or degenerative tears with mechanical symptoms as they are believed to cause osteoarthritis in long run. Partial menisectomy is the most common procedure for treatment of meniscal tears. Though it has good short term results, the long term results of procedure have shown a high progression to osteoarthritis [18]. In another study it proved to be of no benefit in degenerative root tears [19]. Current view is that partial menisectomy should be a very limited procedure and avoided as far as possible even in degenerative meniscal lesions. It can be done in a small subset of patients with irreparable degenerative tears causing mechanical block [20].

Meniscal repair: The first open repair of meniscus was reported by Annandale. Era of arthroscopic repair started when first arthroscopic meniscal repair was done by Ikeuchi. arthoscopic repair predominates the open repair now a days. Open repair is occasionally used particularly if posterior meniscal tears in a very tight medial compartment need to be repaired. the long term success of meniscal

repair is between 70 to 92%. Eggli et al reported successful outcome in 73% after follow up of 7 and a half years. Favourable factors for outcome were injury duration less than 8 weeks, peripheral tears, a small tear of less than 2.5cm, age less than 30 years. Lateral meniscus had better outcome than medial meniscus. the most important condition for good recovery is a stable knee, unstable knee often leads to failures. Suturing techniques for repair of tear can be inside out, outside in or all inside.

Inside out suturing: It is strongest out of the three. It is suitable for anterior and middle 1/3 section of the meniscus. Tear is fixed by placement of sutures from intra-articular region with help of special cannulae to extracapsular area.

Outside in technique: In this technique sutures are passed through two spinal needles from the meniscal body to meniscal rim, the two ends of the passed suture are then tied over the capsule.

All inside technique: This is done with the help of special implants called fixators. These can be arrow head, hook devices, staples or anchors (Fig. 2). These impants are made of polylactic acid and cause implant induced synovitis, which limitis their use. Flexible, suture based implants are more commonly used now. advantages of these all inside implants is that they are technically less demanding and quick, but they have less strength than the sutures.

Besides these repairing techniques, certain procedures have been described in literature to augment healing in meniscal tears particularly in avascular areas.

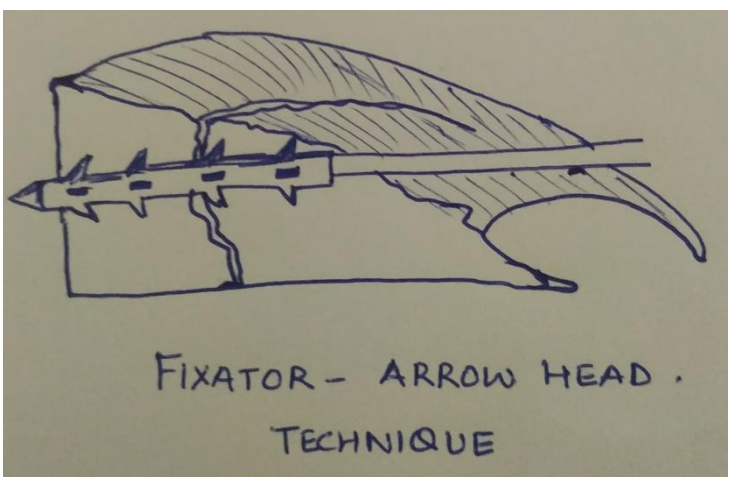

Fig. 2.

Trephination technique: it involves making radial holes in meniscus to aid ingrowth of vascular tissue. Combined with suturing it shows improved results.

Synovial abrasion: abrading synovial tissue around meniscal repair area will increase vasculature and improve healing.

Fibrin clot technique: Patients venous blood is mixed with a glass baguette, this paste is then kept in between torn edges, this has a positive effect on healing due to chemotactic and mitogenic factors.

Meniscal reconstruction: These are the procedure which are used to replace a partially or totally resected meniscus. Though preservation is currently the main aim of treatment, sometimes due to irrepairable injury or previous surgery reconstruction remains the only option. The two procedures for reconstruction are meniscal scaffolds or meniscus transplantation.

Meniscal scaffolds: These are used to fill the defects in resected meniscus by allowing growth of vasculature and cell migration to form meniscal tissue. Two main types of scaffolds are the collagen meniscus implant and urethane scaffold.These are cell free and biodegradable scaffolds. Favourable reports regarding their clinical efficacy and long term results have been reported. lately cell scaffolds have been introduced.

Meniscal transplantation: To prevent arthritis is young patients with a deficient meniscus, transplantation remains an option. Experimentally autografts, allografts, xeno grafts and synthetic implants have been used. The benefit of these in preventing osteoarthritis in long term is still questionable. Allograft transplantation has shown good to excellent results in 84% cases in a meta analysis in athletes. These procedures have favourable outcome only if articular cartilage is smooth and body mass index in less than 30. Discussions on transplantation still continue with good short term and medium term results.

7. REHABILITATION AFTER MENISCAL REPAIR

Two protocols have been followed in the rehabilitation of patient with meniscal repair: *The conservative protocol* and *the aggressive protocol*. In conservative protocol 6 weeks of partial loading , slow increase in knee movements in controlled brace and avoiding sports for 6 months is followed, while as aggressive protocol allows immediate loading and unlimited knee movements and return to sports as long as patient can tolerate. In a comparative study there was no difference in failure rates of the two methods. However result vary on lot of factors besides the rehabilitation protocol, it becomes impossible to compare results of various series. Most surgeons follow the conservative protocol.

8. CONCLUSION

Meniscal injuries are one of the common reasons for knee pain and disability. Proper clinical evaluation is needed to decide best possible treatment for a particular patient. Conservative treatment usually suffices in degenerative tears without mechanical symptoms. Treatment should be aggressive in young athletic

patients who have a fresh injury. Current treatment focuses more on preservation of the meniscal tissue. Focus should be on repair rather than removal.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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Chapter 8
Print ISBN: 978-93-5547-742-2, eBook ISBN: 978-93-5547-749-1

Current Updates on Migrane: Diagnosis and Management

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DOI: 10.9734/bpi/cpms/v9/3460A

ABSTRACT

Migraine is a complex, less well understood neurovascular disorder and it is not just a headache. It presents with a myriad of symptoms due to the pathophysiological processes uniting cortical depolatization, brainstem dysfunction, meningeal vasodilatation and excitation of sensory pain structures as remote as the cervical nucleus caudalis. In the management of migraine, patients are usually in the practice of use of medications purchased over the counter including compound analgesics, triptan or ergot derivatives. Most of the physicians commonly encounter patients with headaches. Hence a rational manner of approaching the patient with these conditions, allows a specific diagnosis of migraine to be made quickly and safely. In this chapter we have attemptd to explain the less understood aspects of this disorder including pathogenesis, management and latest updates regarding treatment.

Keywords: Migraine; neuralgia; headache; aura.

1. INTRODUCTION

Headache disorders are one of the most common ones affecting the nervous system, leading to disability and morbidity in a major population of the world. It remains uncared and undertreated and hence a thorough understanding to unravel the science behind is in need. Furthermore in the developing countries, due to lack of/limited financial resources combined with remote accessibility to rural areas there is found to be extensive need for the systematic collection of information [1]. Headache accounts for 4.4% of all consultations in general practice, approximately 5% of all medical admissions to hospital [Weatherall,

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2006], and approximately 20% of neurology outpatient consultations [2]. Headache is classified as primary, secondary and other neuralgias according to the International Headache Society [3] (Table1).

2. MIGRAINE

Among all the type of headaches migraine is one of the, ubiquitous and essentially treatable. However it also remains less understood, underestimated and undertreated one [4]. It has been termed the seventh disabler due to its considerable impact on the quality of life (QOL) of patient [5]. Migraine affects over 20% of people at some point in their lives; epidemiological studies have shown that 4.5% of the population of Western Europe has headache on at least 15 days per month [Welch and Goadsby, 2002]; global studies suggest that approximately 1% of the world's population may have chronic migraine.. The fact that this disorder is chronic in nature has provoked us to further have a deep understanding of the pathophysiology, investigations and management strategies [2].

Table 1. Classification of Headache disorders

Type	Symptoms
Primary	1. Migraine
	2.Tension type headache
	3.Cluster headache and trigeminal autonomic cephalgias
	4.Other primary headaches
Secondary	5. Headache attributed to head and/or neck trauma
	6 .Headache attributed to cranial or cervical vascular disorder
	7. Headache attributed to non vascular cranial disorder
	8. Headache attributed to a substance or its withdrawal
	9. Headache attributed to infection
	10.Headache attributed to disorder of homeostasis
	11. Headache or facial pain attributed to disorder of cranium, neck, eyes, ears, nose, sinus, teeth, mouth, or other facial or cranial structures.
	12.Headache attributed to psychiatric disorder
Neuralgias and other headaches	13. Cranial neuralgias, central and primary facial pain and other disorders
	14. Other headache, cranial neuralgia, central or primary facial pain

3. PATHOPHYSIOLOGY

3.1 Genetic Basis of Migraine

As far as researches and studies suggest, it is commonly observed that migraine tends to run in families and atleast 50% migraineurs have parents affected by the disorder. The prevalence rate is found to be 1-4% in the male population , 3-6% in the female population with the maximum age being affected being from 22 –55

years. It is statistically estimated that about 30% of patients have suffered from misdiagnosis and hence inappropriate therapy [6]. In addition, family and twin studies support the idea of Migraine without aura and Migraine with aura being different phenotypes of the same entity, with a heritability ranging from 33% to 57% [7].

3.2 Familial Hemiplegic Migraine

Familial hemiplegic migraine (FHM) is a rare subtype of migraine with aura which is found to have an autosomal dominant mode of heritance. The symptoms include hemiparesis, during the aura phase and the aura is generally more prolonged and consists of temporary visual changes such as blind spots (scotomas), flashing lights, zig-zagging lines, and double vision. Usually familial hemiplegic migraine is associated with first or second degree relative with history of migraine. Sporadic hemiplegic migraine is classified as cases with absence of first or second degree relative with history of migraine [8].

Mutations in three different genes have been identified in FHM families: CACNA1A gene, involved in FHM type 1 (FHM1), localised to chromosome 19p13, encoding for the α1A subunit of the neuronal voltage-gated calcium channel Cav2 (neuronal P/Q-type calcium channel) ; ATP1A2 gene localised to chromosome 1q21-23, implicated in FHM type 2 (FHM2) encoding for the α2 subunit of the Na+ K+ - ATPase isoform ; and SCNA1A gene localised to chromosome 2q24, implicated in FHM type 3 (FHM3), encoding a neuronal voltage-gated sodium channel [9]. However newer mutations in further fourth and fifth genes has been found to cause pure hemiplegic migraine [8]. Specifically the genes CACNA1A, ATP1A2 and SCNA1A are found to produce proteins that forma a common ion transport channel that regulate the flow of ions across the neuronal and glial cell membranes. The three genes namely, CACNA1A, ATP1A2 and SCNA1A are found to produce specific proteins, which in turn form channels and these channels form ions which are transported along the glial and neuronal cell membranes. The common phenomenon of ion transport is the one which binds to the three proteins. The uninterrupted smooth flow of ions is responsible for the normal physiological functioningof the neuronal system [8].

It is found that the physiological function of the CACNA1A is to promote influx of calcium ions into the excitable cells [10]. Mutations in this gene, in addition to causing FHM 1 also are found to be contributory to cerebellar ataxis, epilespsy, episodic ataxia type 2 and spinocerebellar ataxia type 6 [8]. ATP1A2, the gene whose mutation is responsible in causing FHM type 2, is found to be mainly expressed in the astrocytes. This mutation is found to be associated with higher resting potential [11]. Mutations in the SCNA1A gene associated with FHM 3 gene, is also found to cause generalized epilepsy with febrile seizures and severe myoclonic epilepsy of seizures [12](Table 2).

3.3 Vascular Theory

The aura of migraine is thought to be generated by cerebral vasoconstriction and headache by relative vasodilatation. On the historical grounds, it was Thomas

Willis who proposed that the theory of intermittent vasodilatation of cerebral and meningeal arteries and Graham and Wolff propounded that migraine is a vascular event with initial vasoconstriction followed by rebound vasodilatation. Amin et al in 2013 identified unilateral attacks of migraine using high resolution Magnetic Resonance Imagimg [8]. Neuropeptides and mediators of inflammation from trigeminal vascular system accompany vascular changes which sensitizes the pain receptors in the peripheral and central neurons in the trigeminovascular system [8].

Table 2. Summary of genes associated with familial hemiplegic migraine [8,10]

Type of Migraine	Gene Associated	Chromosome	Associated Pathologies
FHM1	CACNA1A	19p13	Cerebellar ataxia, episodic ataxia type2,spinocerebellar ataxia type 6
FHM2	ATP1A2	1q23	Mutated ion types in astrocytes have higher potential
FHM3	SCN1A	2q24	Generalized epilepsy with febrile seizures, severe myoclonic epilepsy of infancy

Migrainous fortification spectrum is found to be associated with cortical hypoperfusion that begins in the visual cortex and spreads forward at 2 to 3 mm/min [13]. Numerous research and studies conducted recently to prove the vascular theory as a causative factor for migraine have spoken about cerebral microembolism and cortical spreading depression [14]. The earliest and strongest support for this view came from blood flow imaging studies by Olesen and colleagues, which showed that, during aura-like symptoms, slowly spreading oligaemia propagated anteriorly from the occipital pole. This finding was corroborated during a spontaneous migraine attack without aura seen during PET imaging [15] and also by use of magneto encephalography, which showed multiple cortical areas activated in spontaneous and visually induced migraine aura [16].

Cortical Spreading depression (CSD) which explains vascular theory is a slowly propagating wave of neuronal and glial depolarisation that can be evoked in the cortex, cerebellum, basal ganglia, thalamus, and hippocampus [17]. Ischemic/hypoxic episodes are triggered by endothelin -1 induced vasospasm and microinfarcts or aneurysmal subarachnoid haemorrhage [18]. The ischemic episodes arising after any of the above mentioned causes affect recovery; severe episodes eventually lead to terminal anoxic depolarization, as frequently found in prolonged ischemia. CSD is also found to be associated with genetic and environmental factors. As we already know it genetic factors are susceptible to

modulation by endogenous biological and environmental factors such as oestrogen withdrawal, sleep, and stress, and might also be an expression of neuronal network excitability [14].

3.4 Role of Microembolism

The association between patent foramen ovale and migraine is inconclusive and it has been found that conditions like right to left shunt along with atrial septal aneurysm arising as a result of a patent foramen ovale, makes it a putative risk factor for stroke. The same association is beginning to emerge for migraine with aura [19] Due to increase in the right arterial pressure, in the right to left shunt, transit of fibrin rich, soft, red type venous thrombi occur [20]. Venous emboli are more susceptible to fragmentation, and the resulting small emboli occludes the microcirculation resulting in brief episodes of transient hypoxia/ischemia. In addition the emboli might also activate the platelet system, resulting in the release of pro-inflammatory mediators that might contribute to ischemia [21].

3.5 Role of Large Blood Vessels

As much as microembolism, injuiry to the large blood vessels also have been implicated as a causative factor in migraine with aura [22]. For instance in the event of a carotid artery dissection, a distal embolism might occur as a result of narrow, irregular arterial lumen in the dissected segment [23].

4. ROLE OF NEURAL CIRCUITS IN MIGRAINE

4.1 Trigeminovascular System

The trigeminovascular system carries nociceptive input from the innervated intracranial structures to the brainstem by way of the trigeminal spinal tract of nucleus [24]. On stimulation by neural (electrical) and chemical (neurotransmitters) systems a cascade of events takes place that results in migraine pain. In the mechanism of neurogenic inflammation, on stimulation, the C fibres of the trigeminal nerve start to antidromically release substances such as tachykinins, substance P and Calcium Gene Related Polypeptide (CGRP) into the dural and meningeal blood vesselswhich degranulates the mast cells, releasing histamine causing vasodilatation and plasma extravasation into the tissues. The release of substance P also results in inflammation and swelling of the blood vessel walls [25]. Release of serotonin, local inflammation and the resultant sensitization of polymodal nociceptors by substance P are thought to cause the pain associated with migraine [26].

4.2 Role of 5 Hydroxytryptamine

A major advancement in the understanding of migraine has come with the knowledge of 5 hydroxytryptamine receptors (5HT) [27]. The 5HT1d receptors are involved in the constriction of cerebral blood vessels and arteriovenous anastomoses [28]. The 5HT2 receptor mediates smooth muscle contraction in

many vascular beds through serotonin stimulated release of prostacyclin and products of arachidonic acid metabolism. Theoretically, 5HT2 antagonists are able to inhibit serotonin from inducing an arachidonic acid–derived inflammatory state [24].

4.3 Role of the Autonomic Nervous System

The parasympathetic fibers arise in the superior salivatory nucleus of the brainstem and accompany the facial nerve to traverse the sphenopalatine ganglion and otic ganglion on to the intracranial vessels, including the internal carotid artery. (Ref Fig. 1).

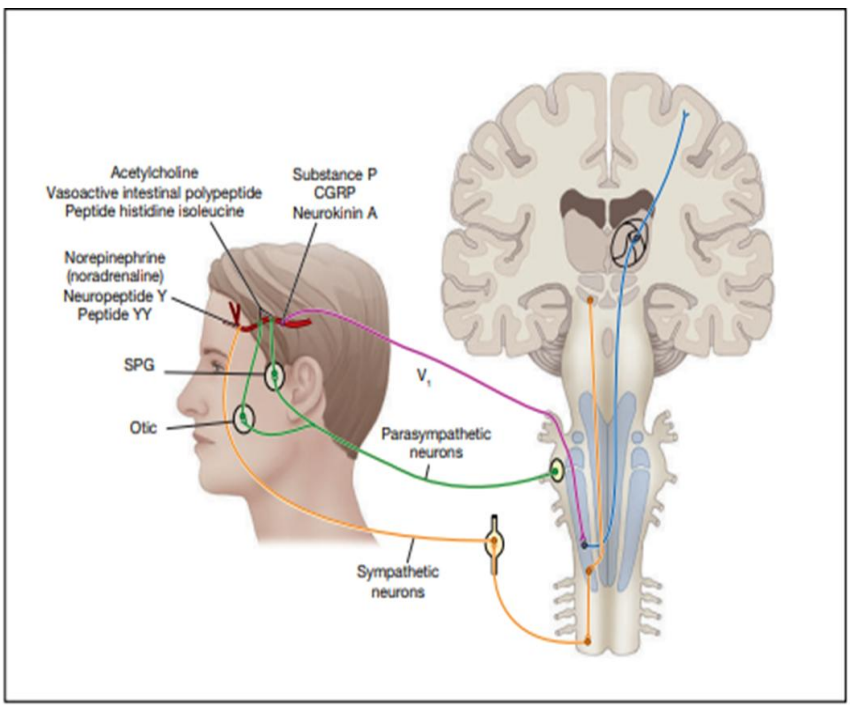

Fig. 1. The central pain pathways and neurotransmitters with afferent and efferent pathways in the trigeminovascular system. (Ref: Bell's Textbook of orofacial pain, seventh Edition)

The parasympathetic fibers are thought to contribute to migraine by the release of certain neurotransmitters like substance P, vasoactive intestinal polypeptide and peptidine histidine isoleucine. These sympathetic fibers can contribute to migraine by the release of norepinephrine, neuropeptide Y, and peptide Y [24].

4.4 Neurophysiology of Migraine

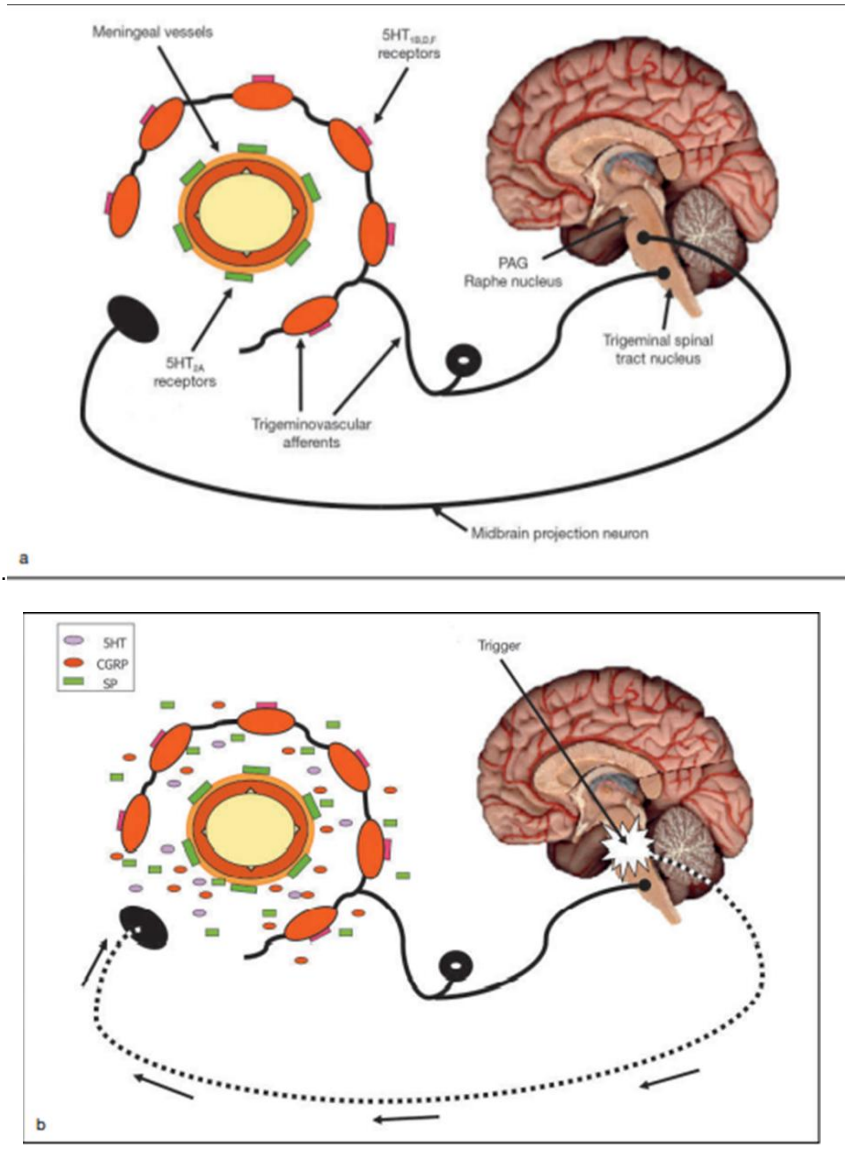

Fig. 2. Graphic description of neurophysiology of migraine [24]

(a) The important anatomical structures involved in producing the migraine include the trigeminal spinal tract nucleus in the brainstem, the midbrain

5HT projection neurons, and the meningeal vessel innervated by the primary afferent neuron. PAG, periaqueductal gray.

(b) Fig 3: A trigger is initiated in the brainstem that causes the antidromic release of neuroactive chemicals such as substance P (SP), CGRP, and serotonin (5HT) into the tissues of the meningeal vessel.

Role of thalamic and cortico thalamic circuits: Studuies show that changes in thalamic and thalamo-cortical activity play a key part in the aberrant sensory processing and could represent a therapeutic target for pharmacological and neuromodulatory approaches such as transcranial magnetic stimulation [29]. Altered network connectivity involving the thalamus, hypothalamus, brainstem, amygdale and cerebellum with consequent overlapping circuits results in alteration of pain and sensory circuits that occurs both locally and interictally in patients with migraine [30]. Cognitive dysfunction is another common symptom of migraine and a cause of disability associated with migraine attacks [31] that could be related to disruptions of normal brain functional connectivity.

5. CLINICAL FEATURES OF MIGRAINE

5.1 Stages of Migraine

Blau [32] has divided the migraine attack into five phases: the prodrome, the aura, the headache itself, the headache termination and the postdrome. The prodrome is the time that occurs hours or even days before the headache is felt. It can consists of mental symptoms like depression, euphoria, irritability, restlessness, mental slowness, hyperactivity, fatigue and neurologic symptoms like photophobia, phonophobia and hyperosmosis. Other general sumptoms may include stiff neck, diarrhea, constipation, fluid retention and food cravings [24].

5.2 Subtypes and Subformes of Migraine (Table 3)

Table 3. Subtypes and subforms of Migraine [33]

Migraine without aura
Migrainew ith aura • Typical aura with migraine headache • Typical aura with non migraine headache • Typical aura without headache • Familial hemiplegic migraine • Sporadic hemiplegic migraine • Basilar type migraine
Childhood periodic syndromes that are commonly precursors of migraine • Cyclical vomiting • Abdominal Migraine • Benign paroxysmal vertigo of childhood

Retinal migraines **Complications of migraines** • Chronic migraine • Status migrainous • Persistent aura without infarction • Migrainous infarction • Migraine triggered seizure
Probable migraine • Probable migraine with aura • Probable migraine without aura • Probable chronic migraine

Migraine without aura: Earlier terminology referred to migraine without aura as common migraine [24]. This headache gives a history of disability lasting between few hours to few days, accompanied with gastrointestinal symptoms and heightened special senses [34]. Migraine without aura is a recurring headache lasting 4 to 72 hours and is unilateral, pulsating with moderate or severe intensity; aggravation by routine physical activity and association with nausea, photophobia, and phonophobia [24]. The headache itself typically lasts some hours and is then succeeded by postdromal fatigue, dulled senses, dysphoria or, conveniently termed the 'migraine hangover' [35].

The International Headache Society [3] has laid down certain criteria that must be fulfilled for the diagnosis of migraine without aura:

1. At least five attacks fulfilling criteria 2 to 4
2. Headache attacks lasting 4 to 72 hours (un‐ treated or unsuccessfully treated)
3. Headache has at least two of the following four characteristics: unilateral location, pulsating quality, moderate or severe pain intensity, and aggravation by or causing avoidance of routine physical activity (eg, walking or climbing stairs)
4. During headache at least one of the following: nausea and/or vomiting or photophobia and phonophobia
5. Not better accounted for by another ICHD‐3 diagnosis

Migraine with aura: Commonly called as classic or focal migraine, the aura evolved over time, usually over minutes, with the visual aura being the most important [34]. The aura may also consist of speech and language symptoms, but no motor weakness with complete reversibility. Auras affecting sensation, movement, cognition, vestibular function, or consciousness may be difficult to distinguish from thromboembolism, or from epilepsy (especially occipital seizures). People presenting with recent onset MA often give a longer history of MO, mistakenly diagnosed as "bilious attacks", "sinusitis", or "normal headaches". Visual symptoms are positive (seeing things which are not there), homonymous and binocular, though some patients insist that visual aura is monocular, raising the possibility of retinal origin. Aura typically precedes

migraine headache, though can occur at any time in relation to pain [34]. The visual aura is commonly characterized by a zigzag, flashing colored phenomenon that migrates across the visual field. This is called techiopsia [24]. Negative symptoms such as blind spots, hemi – anesthesia and rarely hemiplegia are known to be associated with spreading cortical neuronal depolarization, which subsequently leads to hyperpolarization. This phenomenon has been demonstrated on PET scans [35].

5.3 Diagnostic Criteria: [24]

1. At least two attacks fulfilling criteria 2 and 3
2. Aura consisting of visual, sensory, and/or speech/language symptoms, each fully reversible, but no motor, brainstem, or retinal symptoms
3. At least two of the following four characteristics: at least one aura symptom spreads gradually over 5 minutes or more and/or two or more symptoms occur in succession; each individual aura symptom lasts 5 to 60 minutes; at least one aura symptom is unilateral; and the aura is accompanied or followed within 60 minutes by a headache
4. Not better accounted for by another ICHD‑3 diagnosis, and transient ischemic attack has been excluded.

Table 4. Some comorbidities associated with migraine [14]

Cardiac and pulmonary:

- Patent foramen ovale (associated with large openings, atrial septal aneurysms and right to left shunting)
- Mitral valve prolapsed
- Pulmonary arteriovenous malformations

Vascular:

- Stroke
- Carotid or vertebral artery dissection
- Carotid artery puncture
- Brain arteriovenous malformation
- Hereditary disorders (CADASIL, Co14A1 mutations,AD-RVCL, hereditary vascular retinopathy)

Inflammatory:

- Raynaud's phenomenon
- Sjogren's syndrome
- Antiphospholipid antibodies
- Coagulopathy
- Thrombocytosis
- Polycythemia vera

6. UNCOMMON FORMS OF MIGRAINE

Basilar Artery Migraine: This is a kind of migraine aura with deficits in the pathway of basilar artery distribution specifically. The symptoms include brainstem dysfunction, including bilateral visual loss, vertigo, dysarthria, ataxia, tinnitus, hearing loss, global parasthesias, altered consciousness, and syncope. Autonomic changes such as flushing, anhydrosis, ptosis, midryasis, pulse and blood pressure changes and diarrhea can occur [36].

Acephalgic migraine: Acepalgic migraine is aura without headache and the incidence increases with age and also as the episode of migraine decreases. They are often described with the typical features of migraine aura, such as visual obscurations in one hemifield, lasting 15 to 30 minutes, but always need further evaluation like an MRI, and EEG because they do raise a red flag as a NEW phenomenon [36].

Table 5. Common migraine triggers [24]

Diet
• Hunger
• Alcohol
• Additives(Nitrites, monosodium glutamate)
• Certain foods(red wine, aged cheese)
Chronobiologic
• Sleep(too much or too little)
• Change in schedule
• Hormonal factors(Menstruation**)**
Environmental factors
• Light(bright, flashing, glaring)
• Odor(perfume, cigarette smoke)
• Altitude(airplane travel)
• Weather changes
Head and neck pain
• Temporomandibular disorders or joint pain
• Cervical myofascial pain
• Toothache
Physical exertion
• Exercise
• Sexual activity
Stress and anxiety
• Increased exposure to emotional stress
• Let down after a stressful period
Head trauma

6.1 Investigations

Goals of Examination: The main goal of examination is to consider structural brain disease, screen for co-morbid disease such as hypertension and depression (neither of which commonly cause headaches), and to reassure the patient, their family [34]. It is important to mention here that almost every case of migraine does'nt need investigation.

History: Explanation of symptoms by patient helps us to establish the duration, frequency and periods of freedom from pain: a diary can be very helpful. Probe the patient carefully about nausea, light, noise sensitivity and also his/her activities during the attack. Whether the patient is able to carry out the usual activities during the dark? [34].

The following three-question screening developed by Lipton et al. can help in diagnosing migraine [37]. In the last three months, did you have, the following with your headaches:

1. You felt nauseated or sick to your stomach?
2. Light bothered you a lot more than when you do not have headaches?
3. Your headaches limited your ability to work, study, or do what you needed to do for at least one day?

If the patient responded positively to two of three questions, they have 93% chances of having a migraine; if all three responses are positive, they have a 98% chance of having migraine headaches [34].

Diagnosis of Chronic Migraine: When assessing patients with chronic headache , typically , two patterns of origin of headache exists. In one set of cases, patients with a pre-existing primary headache disorder (usually, but not exclusively migraine) have ever-increasing attacks until they reach a stage where they do not recover headache freedom in between, a pattern originally called 'transformed migraine' [2].

In the other set of cases, patients start to have a headache one day, and it simply never goes away. This is a syndrome that goes under the name 'new daily persistent headache' (NDPH) [2] and is an important pattern to recognize because it is within this set of headaches that many of the serious causes lie [2].

Recognition of the disorder: Recognizing the pattern of spread of headache is the primary factor which gives us a clue in identifying the disorder. Physicians have to take in account, the genetic basis and also the internal and external influences which might have triggered the attack. The most striking feature of the disorder is the unilateral pattern of spread. Equally commonly, however, pain is felt bilaterally, at the front or the back of the head, more rarely in the face, and rarer still in the body ('migrainous corpalgia'). Many a times, even moderate exertion or slightest movement might serve to worsen the pre-existing pain [2].

Imaging modalities: Imaging is usually only reserved for situations wherein the probability exists of an underlying tumour and the percentage exceeds 1%.

Table 6. Common Imaging modalities [34]

Types of Headache	Mandatory imaging
First, worst, thunderclap headache	CT(emergency)
Exertional, cough or Valsalva headache	MRI
Headache + signs suggesting brain lesion	MRI or CT
Headache + known malignancy	MRI or CT with contrast optional imaging
Ne headache in older person	MRI or CT
Headache not MO, MA, TTH. MOH or cluster	MRI or CT

CT. computed tomography; MA/MO, migraine with aura, without aura; MOH, medication overuse headache; MRI, magnetic resonance imaging; TTH, tension type headache

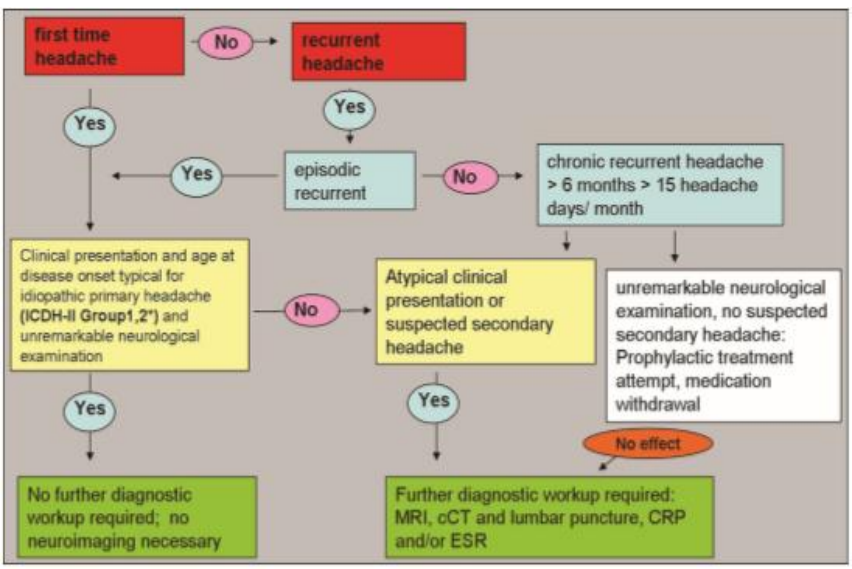

Fig. 3. Flow chart depicting decision making in headaches [38]

Management of Migraine: The management of migraine can be done by many methods which include patient education and trigger avoidance, pharmacological method and non pharmacological methods. Each of the methods have specific effects on the management of the disorder.

Patient education and trigger avoidance: The primary aspect of management of migraine includes patient education about the benign nature of the disorder, however addressing the presence of intracranial tumours identified thorough imaging modalities [24]. Patients must be briefed about the association of pain

and triggers which cause, lest it is frequently overlooked upon. The best way to identify the trigger is to maintain a pain diary. The patient must make it a habit with all sincerity to make a note of the aggravating factors associated with headache , so the it can be avoided as much as possible [33]. When patients have chronic severe headaches, it can be difficult to recognize specific triggers. Paradoxically it is often the case that as chronic headaches start to improve with treatment, triggers become more obvious. Regarding lifestyle modifications, regularity of regimen with regards to meals, sleep pattern and hydration definitely has it's own implications in the management of migraine. Environmental changes like sudden exposure to extreme weather and temperature is remarkable in triggering an attack of migraine [2].

Patients with chronic migraine often have added co morbidities like anxiety, depression and other pain syndromes such as fibromyalgia, localized pain in head and neck structures, and conditions that create 'metabolic' strain such as sleep apnoea or postural orthostatic tachycardia syndrome. In such cases a standardized protocol has to be meticulously formulated for management of patients [2]. The goal of management should be to improve quality of life, improve pain control and allow smooth day to day activities [39].

6.2 Pharmacologic Management

Acute migraine treatments: The usual principal in treating acute headaches include:

I. Attacks should be treated early, when the pain is still mild.
II. Effective doses should be used.
III. Treatments being titrated steadily till the maximum effective dose is reached, before being abandoned
IV. Treatment of associated symptoms including nausea, vomiting etc
V. Determination of the appropriate route of treatment [2]

Table 7. Staged approach to treatment of migraine [13]

Stage	Diagnosis	Therapy
Mild migraine	Occasional throbbing headache; No impairment of daily function	NSAIDs, Combination analgesics, Oral serotonin agonists
Moderate migraine	Moderate to severe headache, nausea, some impairment of functioning	Oral, nasal or subcutaneous serotonin agonists, oral dopamine antagonists
Severe migraine	Severe headache (>3 times / month), marked nausea/vomiting, significant functional impairment	Intramuscular or intravenous dopamine antagonists, prophylactic medications.

Table 8. Line of drugs used in treatment of acute migraine [2]
NSAIDS

Name of drug	Dosage
Paracetamol	1 gm
Aspirin	900 – 1200 mg
Ibubrufen	400 – 800 mg
Naproxen	250 – 500 mg

7. OTHER NON SPECIFIC PHARMACOLOGIC MANAGEMENT OF MIGRAINE [39]

1. Antiemetics

a. Chlorpromazine – IV/IM
b. Prochlorperazine – IV/IM/Per Rectum (PR)
c. Metoclopramide – IV/IM/PR – IM/PR routes have shown inconsistent evidence for effi cacy but may help with gastric paresis, which occurs during migraine, thus improving absorption of other oral medications. It is also used IV with DHE to counteract nausea.

2. NSAIDs and nonnarcotic analgesics

a. Ketorolac IM, IV
b. Oral NSAIDs: aspirin, naproxen, diclofenac, ibuprofen etc are all benefi cial in mild migraine (Diener et al 2004; Lipton 2005).
c. Combination analgesics

i. Acetaminophen, aspirin, caffeine (mild migraine)
ii. Butalbital, ASA, caffeine – has shown inconsistent evidence for efficacy in migraine, mainly studied in tension-type headache.
iii. Isometheptene mucate, acetaminophen, dichloralphenazone

3. Opiate analgesics – to be used judiciously by experienced physicians, primarily as a 'reserve' medication, for acute severe attacks not responsive to selective abortive agents. This can prevent overuse of ER services in patients with difficult to control migraine pain.

a. Butorphanol nasal spray
b. Acetaminophen with codeine, hydrocodone, hydromorphone
c. Oral transmucosal fentanyl citrate

8. GENERAL CONSIDERATIONS IN PHARMACOLOGICAL MANAGEMENT OF MIGRAINE

I. Triptan agents are probably the most effective agents and used over the past 20 years yielding a remarkable achievement in the management of migraine.

Table 9. Currently available Triptans [33]

Triptan	Medication form and strength (mg)	Recommended dosage/interval (>hrs)	Maximum daily dosage (mg/24 hrs)
Almotriptan (Axert)	Tablet 6.25, 12.25	2	25
Eletriptan(Relpax)	Tablet 20,40	2	80
Frovatriptan(Frova)	Tablet2.5	2	75
Naratriptan (Amerge)	Tablet 1, 2.5	4	5
Rizatriptan (Maxalt)	Tablet 5,10	2	30
	Oral disintegrating tablet, 5,10	2	30
Sumatriptan(Imitrex)	Tablet 25, 50, 100	2	200
	Nasal spray 5,20	2	40
	Subcutaneous, 6	1	12
Sumatriptan 85mg+naproxen 500mg (Treximet)	One fixed dose tablet	2	2 tablets
Zolmitriptan (Zomig)	Tablet 2.5,5	2	10
	Oral disintegrating tablet, 2.5,5	2	10
	Nasal spray, 5	2	10

Table 10. Other groups of drugs

Name of drug	Start dose (mg)	Maximum dose (mg)	Side effects
Propanolol	20	320	Cold limbs, nightmares, Atenlol may be better tolerated
Pizotifen	0.5	3	Weight gain, sedation, low efficacy
Valporate	500	1500	Weight gain, teratogenecity, Consider gabapentin, topimarate
Methysergide	1	8	Limb pain, visceral fibrosis, 6 montha treatment, 1 month break

Table 11. Management of chronic migraine [2]

First line	Starting dose	Target dose
Beta blockers		
Propranolol	10 mg three times daily	40 – 80 mg three times daily
Metoprolol	25 mg twice daily	100 mg twice daily
Atenlol	25 mg once daily	100 mg oncedaily
Angiotensin blockers		
Candesartan	4 mg once daily	12 – 16 mg once daily
Tricyclics		
Amitryptiline	10 mg nocte	75 - 100 mg nocte
Nortriptiline	10 mg nocte	75 – 100 mg nocte
Dosulepin	25 mg nocte	75 – 100 mg nocte
Second line		
Anticonvulsants		
Topimarate	12.5 mg nocte	50 – 100 mg twice daily
Sodium valporate	200 mg nocte	400 – 800 mg twice daily
Flunarizine	5 mg once daily	5 – 10 mg once daily
Supplements		
Riboflavin (Vit B2)	400 mg daily	
Magnesium citrate	600 mg daily	

II. All the triptan agents are serotonin (5HT 1B and D agonists) and cause a notable degree of vasoconstriction in the meningeal vessels and other vascular beds, thus eliminating the vasodilatation involved in causing migraine [40]
III. In a patient who is vomiting, oral agents are replaced with nasal spray, injections or patch.
IV. NSAIDs and Tylenol can be safely used as adjuncts along with triptans [40].
V. Side effects of triptan include tingling, flushing, sensation of warmth, pressure, tightnessin different parts of the body and in rare instances myocardial ischemia [33].
VI. Dihydroergotamine is a traditional drug and one-half to 1 mg can be injected subcutaneously, intramuscularly, or intravenously, and the dose can be administered a total of three times in 24 hours [33].
VII. In addition there are also many over the counter drugs used which include nonsteroidal anti-inflammatory drugs (NSAIDs), acetaminophen, weak and strong opioid analgesics, and combinations of these drugs. Triptans also can be combined with another analgesic such as a NSAID [33].

9. OTHER NON PHARMACOLOGICAL METHODS OF MANAGEMENT OF MIGRAINE

Greater occipital nerve blockade: The greater occipital nerve is found to arise in close proximity to the trigeminal afferents and it's blockade is found to be reasonably effective [41] but an invasive procedure. This blockade is primarily indicated in patients with occipital tenderness [42].

Onabotulinimtoxin A: The efficacy of onabotulinimtoxin A was first demonstrated in a open label study [43]. In chronic migraine, which is defined as headache on ≥15 days per month for ≥3 months, of which ≥8 days, Onabotulinimtoxin A has a role to play [44]. Long-term data also suggest that most of those who initially respond will continue to do so for at least two years [45].

Neurostimulation: Transcranial magnetic stimulation (TMS) is found to interrupt spread of cortical spreading depression by delivering a fluctuating magnetic field from the scalp [46]. Many studies have proved that single pulse TMS is effective in treating acute migraine and repetitive TMS has a role to play in migraine prophylaxis [47].

Supraorbital Transcutaneous stimulator: Nerve stimulation involves external application of electric current in abolishing pain signals. Various studies showed that it decreased pain in more than 50% of patients [48].

Non Invasive Vagal Nerve Stimulation: The benefits of vagal nerve stimulation (VNS) in treating migraine attacks were incidentally noted while treating patients with intractable epilepsy [49]. Gammacore® is a portable non-invasive VNS

(nVNS) that transmits a small electrical signal to the vagus nerve through the skin when held against the neck [50].

Calcitonin Gene Related Peptide antagonists: These are a novel group of drugs with a promising anti migraine effect. Two recently introduced are: BIBN4096BS and Compound 1. BIBN4096BS which are found to inhibit vasodilation [51]. Intra venous and Oral administration of CGRP receptor antagonists (olcegepant and telcagepant), 'gepants', were designed for relieving pain during acute migraine and they showed similar efficacy to triptans [52]. Olcegepant is administered intravenously, whereas telcagepant is administered orally .However hepatotoxicity a serious adverse effect has forced it's withdrawal [53].

New routes of administration: Adminstration of drugs through inhalation (nasal form also) and transdermal delivery systems are promising. Another device is being investigated which will soon start phase II investigation which includes sending pressurized carbon di oxide through the nostril [39].

10. CONCLUSION

Migraine is an undertreated headache and is managed by over the counter drugs to provide relief at times of episodes without exploring into the basic pathology associated with it. Judicious use of the drugs at instances has a significant impact in reducing the discomfort and disability associated with migraine. Patients education about triggers associated with migraine and background information about the line of drugs commonly used to manage the disorder remain the mainstay of getting over the disorder. Our existing armamentarium holds plenty of possibilities for clinicians and patients to work together to improve the lives of people with migraine.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Chapter 9
Print ISBN: 978-93-5547-742-2, eBook ISBN: 978-93-5547-749-1

Quality Assessment of Clinical Practice Guidelines (CPG) for Management of Paediatric Dental Emergencies Applicable (PDEA) during COVID-19 Pandemic

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DOI: 10.9734/bpi/cpms/v9/16400D

ABSTRACT

The present study evaluates the quality of Clinical Practice Guidelines (CPG) related to the management of paediatric dental emergencies applicable to the COVID-19 pandemic, through the use of the measuring instrument AGREE II (Appraisal of Guidelines for Research and Evaluation in Europe).

We conducted a thorough web search of CPG among the top CPG compilers. : In addition, a thorough search was conducted among the major national and international dental organisations as well as reputable websites in order to find CPG that fulfil the inclusion criteria.

An overall Five (05) out of twenty-three (23) selected CPG, were classified as "acceptable" according to AGREE II. These five guides were evaluated to determine their "Recommendation degree". According to the quality evaluation criteria and recommendation degree of the AGREEII instrument, only one CPG (AUGE clinical guide for ambulatory dental emergencies- Chile, 2011) was considered a "Rec-ommended" CPG, but applicable only among Spanish-

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speaking coun-tries. It would be advisable to work on this guide, using English as an international language.
High, middling, and low quality CPG were determined using the quality assessment and recommendation degrees criteria from AGREE II. Only one CPG had a score of 75% or above, earning the designation "highly recommended". Therefore, in order to ensure their quality, it is advised that both current and future CPG updates develop them using the available tools and procedures.

Keywords: Dentistry; health profession; emergency medicine; pediatrics; clinical practice guide; quality; guidelines; AGREE II; COVID-19.

1. INTRODUCTION

Due to the breakout and spread of the new SARS-Cov-2 (Severe Acute Respiratory Syndrome), which was first discovered in the Chinese city of Wuhan in December 2019, the world's public health is currently experiencing a serious crisis [1]. The World Health Organization (WHO) [2] renamed COVID-19 and classified it as a pandemic on March 11th, 2020 [3]. COVID-19 is characterised by symptoms including fever, cough, fatigue, myalgia, dyspnea, and occasionally diarrhoea. The majority of those at risk are elderly people and patients with co-morbid conditions like hypertension, diabetes, and obesity. On the other hand, the majority of COVID-19 paediatric patients exhibit mild symptoms, no fever nor pneumoniae. During the first phase of the pandemic, there were not severe cases or deceases reported among paediatric patients [4]. As a matter of fact, a study that analysed 44,672 confirmed cases in China since February 2020, reported that only 416 cases (0.9%) were patients under 10 years old [5]. By June 2020, only two (02) deaths in children testing positive for COVID-19 were reported in China and no deaths, in Italy (the two countries with more confirmed cases). Nevertheless, with the progressive increase of confirmed cases in the adult population, the number of paediatric infections also increased concomitantly [6]. Dental procedures can generate large numbers of droplets and aerosols [3], and studies have shown that the virus can stay alive in aerosols for up to 3 hours after treatment; on surfaces, the virus can remain alive for days [7,8]

In general, any patient (either adult or paediatric) ought to be considered as potential COVID-19 carrier [9]. A large percentage of COVID-19 confirmed cases are asymptomatic or have mild symptoms [9-11]. Wang et al. identified some risk factors associated with the virus transmission during dental treatments in paediatric patients i.e. the droplets emitted during sneezing and the aerosols generated by the high-speed piece [12]. The American Dental Association (ADA) and the Centres of MediCare and MedicAid Services (CMS), recommend that during the pandemic, dental procedures should be restricted only to emergencies so as to reduce the risk of virus spread among patients and dental staff [13,14]. Several formal adaptation approaches are currently available, and they may be tailored further to suit particular circumstances. Evaluations like the one described in the present study should provide guidance for appropriate CPG

adaptation or development efforts, particularly for organizations with little expertise with the AGREE II instrument [15].

Emergency dental care is necessary in cases of life-threatening tissue bleeding, excruciating pain, or serious infection. Contrarily, urgent dental treatment is concentrated on treating diseases that must be treated right away in order to relieve moderate to severe pain, lower the risk of infection, and lessen the patient load in emergency facilities [14].

The most frequent emergencies in children are: the reversible pulpi-tis, irreversible pulpitis [16], acute apical periodontitis, facial cellulitis, facial abscess and dental trauma [17]. Half of them are characterised for presenting sequelae related to dental caries [18].

The management of dental emergencies has become increasingly important due to the COVID-19 pandemic. The constant search for reli-able scientific evidence, that allows solving clinical doubts and identi-fying suitable treatments, is more frequent in this context. As a result, it is necessary for the clinical dentists to have access to high quality CPG, which enable them to promote and recommend practical solutions to clinical doubts regarding efficient treatments in their daily routines. CPG can represent one part or the determining pillar in the elaboration of health policies. Therefore the preparation of CPG requires rigorous methodologies to ensure its quality. However, we need to take into consideration that not all CPG meet the basic requirements [19, 20].

AGREE II is a reliable tool which assesses the methodological rigour and transparency used in the CPG preparation [21]. After having used this practical tool, it was shown that some CPG did not present an adequate structure, either due to a poor quality elaboration or a lack of updated scientific evidence [22]. The quality of a CPG is defined as the confidence that potential biases (in the development of the guide) have been adequately pointed out and that the recommendations are valid, both internally and externally [23].

The purpose of this systematic review is to evaluate the quality of the CPG for the management of paediatric dental emergencies, published in the period 2000–2020 and applicable to the context of pandemic, by using the AGREE II tool. Additionally, to provide relevant information to those researchers and/or institutions responsible for the development of CPG worldwide.

2. METHODS

The present systematic review was registered in PROSPERO (registry number: CRD42020195678) and detailed methods are available in the published protocol [24]. The systematic review is reported according to PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) and to a checklist available as supplementary material [25]. The protocol was focused on the strategic search for published and available CPG. The questions, asked for the present review, were:

How many CPG for the management of paediatric dental urgencies and emergencies, are available and applicable to the COVID-19 pandemic?

Which high quality CPG could be recommended?

Details regarding the search are visualised in Fig. 1.

2.1 Inclusion and Exclusion Criteria

Inclusion criteria:

- CPG published in databases and gray literature, aimed at dental emergencies in children, and applicable to the current context of the COVID-19 pandemic.
- CPG written in any language.*
- CPG published between 2000 and 2020.

* The native language of the evaluators is Spanish, with basic knowledge of English, Portuguese and Italian. For other languages, translations tools were used (Available:https://www.enago.com/ar/y Available:https:// oxfordediting.com/). The CPG, included in this study, and the AGREE II instrument were translated into Spanish. Table 1.

Exclusion criteria:

- GPC that do not contain full text.
- Previous versions of GPC
- GPC aimed at children with special abilities.
- CPG aimed at adults.

Ethical approval and informed consent were not necessary since no human beings were involved.

2.2 Search Strategy

An online search was carried out among the main CPG compilers: National Institute for Health and Clinical Excellence (NICE), National Guidelines Clearinghouse, Agency for Health Research and Quality (AHRQ), Andalusian Health Technology Assessment Department (AETSA), American Academy of Family Physicians and Tripdatabase. The key terms used for this search were: (Guide practice dental emergency children), (guidelines emergency dental), “urgency dental”, (guidelines dental urgency emergency children); associated with the boolean oper-ators: “AND” and “OR”. This search was carried out from 30th of April to 30th of July, 2020.

Additionally, a manual search was carried out for CPG that met the inclusion criteria and were available on the websites of various national and international dental organizations.

2.3 Screening and GPC Selection

Initially, 5070 articles and CPG were collected. After the first filter, carried out by the reviewer “ASA”, and the subsequent examination carried out by the reviewers “JAM” and “GPS”, 5026 guides were excluded as they did not contain eligible aspects in the title and/or ab-stract. As a result, 44 CPG were selected for further content evaluation. Subsequently, a videoconference with all the reviewers (“JAM”, “ASA”, “GPS”, “GCHS”, “RLV” and “GTR”) was held to support the inclusion or exclusion of the assigned documents. Any disagreement among the re-viewers was solved with further discussion and in cases where consensus was not reached, the judgement of an expert reviewer (“GTR”) was decisive. Eventually, only 23 papers met all the selection criteria. These were processed for data extraction and quality evaluation Table 1.

2.4 Data Extraction

The 23 selected CPG were assigned to the reviewer “JAM” to sort them according to their characteristics (year of publication, origin, type of guide – Expert opinion, Consensus or Based on evidence) and to classify them according to their specialty (dental emergencies) Table 2.

The evaluation of the quality and recommendations was carried out by using AGREE II.

2.4.1 AGREE II

AGREE II, an instrument for evaluating research guidelines, is commonly used to evaluate the quality of the information of the studies (components of the preparation and documentation of the process) and the recommendation degrees [23].

It is worth highlighting that this instrument does not contain specific criteria to assess the quality of clinical contents, nor the evidence that supports it.

Currently, this instrument is available in several languages and it also has a training manual which is aimed at guiding those who wish to critically evaluate any CPG [26]. Available:Available:https://www.agreetrust.org/resource-c entre/

The AGREE II instrument consists of twenty-three [27] items, orga-nized into six (06) domains, followed by two (02) global scoring items (General quality of the guide and Recommendations for its use). Each domain represents a unique dimension for quality evaluation, with a specific score that will determine whether the guide should be used (recommended) or not. All of the AGREE II items are ranked using a Likert-type scale from 1 (strongly disagree) to 7 (strongly agree). Available:Available:https://doi.org/10.1371/journal.pone.0174831.t001.

2.4.2 Analysis with AGREE II

The 23 selected guides were independently reviewed and five (05) CPG were classified as “acceptable”. After this result, all of the reviewers were calibrated (trained) in the use of this tool by an expert reviewer (“GTR”) via online (Cisco Webex and Zoom).

An instructive guide was used for this training. Subsequently, the evaluation of all of the five CPG was completed by each reviewer, who presented their data independently. The data were assessed for statistical analysis by using Cohen Kappa coefficient. The objective: to determine the degree of concordance among the reviewers.

During the calibration/training period, any discrepancy among the reviewers was discussed until consensus was reached and the Cohen Kappa coefficient (0.8) was obtained. All the data were compiled in a single table, in alphabetical order according to title, country of origin, organization that prepared it, year of publication etc. In addition, the evaluation scores achieved by all the CPG, according to the 6 domains of AGREE II, were included.

The Recommendation Degrees (RD) of the selected CPG were deter-mined by using the following strategy: The guidelines could be classified as “Recommended” (R) (when at least 3 domains are 60%) “Recom-mended with Modification” (RM) (>30% to <60%) and “Not Recom-mended” (NR) (when at least 3 domains are ¼< 30%) [28]. Table 4.

The domain scores were calculated by adding the scores for each item in that domain and then scaling the total, as a percentage of the “Maximum Possible Score” for that domain. This was carried out by using the following mathematical operation:

Score obtained – Minimum Score ¼ Percentage for that domain.

Maximum Possible Score – Minimum Possible Score x 100.

All this was weighted by the 4 reviewers, who qualified as concordant according to Cohen Kappa Coefficient Fig. 2.

2.5 Statistical Analysis

The statistical analysis was carried out by using Stata V.15 software (Stata Corporation, College Station, Texas, USA).

The concordance degree among the reviewers for the eligibility of the guideline was calculated by using Cohen Kappa Coefficient, a qualitative assessment. (Cerda, L et al. 2008). [27]. The Kappa concordance coeffi-cient among the 4 reviewers was k ¼ 0.82. In addition to this, “Generalization of weighted Kappa coefficient” for more than two observers was necessary. After downloading the

command Kappa2 (through the syntaxes “findit kappa2”) the expression “kappa2 OB01-OB04, wgt(w2)” was executed. All this, in order to obtain the global result.

The characteristics of the CPG were summarised by using descriptive statistics. The general scores of the included CPG are presented for each AGREE II domain through summary measures (mean, median and stan-dard deviation) and Shapiro-Wilk p.

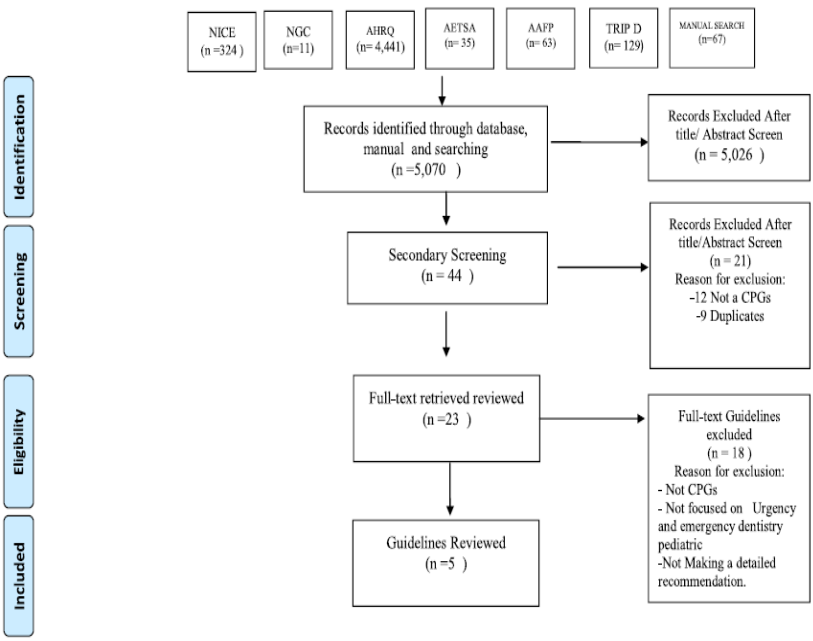

Fig. 1. Guide identification and recovery flow chart

3. RESULTS

From the analysis of the 23 CPG selected according to the inclusion criteria, the following results were obtained: A gradual increase in the number of CPG publications was observed over the years. Of the total selected CPG, 13% of them were published between 2000 and 2010, 26.1% were published between 2011 and 2015; and 60.9%, between 2016 and 2020. 21.7% of the selected CPG came from Europe, 4.3% from Asia and 73.8% from America. On the other hand, five (05) were considered specific for paediatric dental emergencies, and applicable to this current context of pandemic. In addition, regarding the method of elaboration, 34.8% (08 CPG) of the selected guides were based on expert opinion, 47.8% (11 CPG) were created with consensus, and 17.4% (04 CPG), based on evidence Table 2.

Table 1. CPG compiler agencies (Search and storage)

Organisms		Electronic address	Keywords	CPG found	CPG excluded	CPG included for your review	CPG excluded after review	CPG included to evaluate with the agree ii instrument	CPG excluded after evaluation with agree ii	CPG included after evaluation with agreii
NICE	NICE	Available:https://www.nice.org.uk/	Guide practice dental emergency children	324	320	4	1	3	0	3
National Guideline Clearinghouse	NGC	Available:https://archive-it.org/collections/5265	Guidelines emergency dental	11	10	1	1	0	0	0
AHRQ Agency for Health Research and Quality	AHQR	Available:https://www.ahrq.gov/	“Urgency dental”	4,441	4,441	0	0	0	0	0
AETSA	AETSA	http://www.juntadeandalucia.es	Guide practice dental emergency children	35	35	0	0	0	0	0
Primary Care Clinical Practice Guidelines	AAFP	Available:https://www.aafp.org/about/policies/all/joint-development.html	Guidelines dental urgency emergency	63	63	0	0	0	0	0
TripDatabase	TRIP D	http://www.tripdatabase.com	Guidelines dental urgency emergency children	129	127	2	0	2	1	1

Organisms		**Electronic address**	**Keywords**	**CPG found**	**CPG excluded**	**CPG included for your review**	**CPG excluded after review**	**CPG included to evaluate with the agree ii instrument**	**CPG excluded after evaluation with agree ii**	**CPG included after evaluation with agreii**
MANUAL SEARCH	Google Scholar	Available:https://scholar.google.es/schhp?hl = es	Guidelines dental urgency emergency	67	30	37	19	18	17	1
TOTAL OF GUIDES				5,070	5,026	44	21	23	18	5

The global evaluation of the 23 selected CPG, revealed that 78.3% (18 CPG) were "Not recommended" (NR) due to the lack of methodological rigour. Moreover, 21.7% (05 CPG) were identified to have acceptable quality and were categorised as "Recommended" (R) or "Recommended with Modification" (RM). On the other hand, regarding the Evaluation of the domains" in all the 23 CPG, it was shown that Domain I "Scope and Purpose" was the only one obtaining the highest average score (39.3%) and the Domains III and V (Rigour of Development and Applicability, respectively) obtained the lowest scores. The Shapiro-Wilk statistical test showed that Domains III, IV and VI (in all the 23 selected CPG) presented a statistically significant difference ($p < 0.01$) Table 3.

After evaluating all the domains in the 5 CPG classified as "accept-able" for this review, the following results were observed: The domain that achieved the highest average score was Domain I "Scope and Pur-pose" (76%) and the one with lowest score was Domain V "Applicability" (24%). On the other hand, Domain III, corresponding to "Rigour of development", ranged from 38% to 65% with an average score of 44.4%. Furthermore, according to Shapiro-Wilk, it is observed that Domain III, presented $p < 0.01$, indicating that there is a statistically significant difference among the 5 CPG with respect to this domain. The summary measures (Mean, Median and Standard Deviation) were also obtained in each of the domains Table 4.

The quality evaluation of the 5 CPG using the AGREE II domains assessment, revealed that there was no specific CPG for the management of paediatric dental emergencies. However, when the objective of this topic was rigorously evaluated in these 5 CPG, it was observed that only one (AUGE Clinical Guide for Ambulatory dental emergencies – Chile, 2011) [29] reached the highest score (75%). This document exhibited 5 domains with a score 60%, including Domain III, and it was considered as "Recommended", while the other four guides reached an average score of 43.5%. The results of the evaluation of these 4 guidelines were: Scotland, 2013 [30] that obtained 50%; Brazil, 2013 [31] that obtained 45%, Sweden, 2012 [32] that obtained 44% and Italy, 2012 [33], with 35%. All of them presented 1 to 2 domains with a score 60%. Furthermore, they all presented 1 to 2 domains with a score 30%. As a result, these four CPG were categorised as "Recommended with Modifi-cation" Table 4.

4. DISCUSSION

These CPG for the management of paediatric dental emergencies have gained big importance during this COVID-19 pandemic since the American Dental Association (ADA) and the Centres for Medicare and Medicaid Services (CMS) [13,14] recommended prioritising dental emergencies to avoid the spread of SARS COV-2 among patients and oral health professionals. Considering the current global situation we are undergoing, we planned to carry out this systematic review in order to find CPG, based on scientific evidence, with high methodological quality and applicable to this COVID-19 context.

Table 2. Characteristics of the Clinical Practice Guidelines included

Characteristics of the Clinical Practice Guidelines included	Number	Percent
Year of publication		
2000–2005	2	8,7%
2006–2010	1	4,3%
2011–2015	6	26,1%
2016–2020	14	60,9%
Continent of published guidelines		
Europe	5	21,7%
Asia	1	4,3%
North America	1	4,3%
Center America	1	4,3%
South America	15	65,2%
Type of Guideline		
Expert opinion	8	34,8%
Consensus	11	47,8%
Based on evidence	4	17,4%
Guideline specific to dental emergencies?		
Yes	5	21.7%
No	18	78,3%

Using the Agree II tool, we accomplished quality evaluations on all the CPG available online. The results of this review indicated that the general quality of the CPG for paediatric dental emergencies is mainly medium or high. These guides may be recommended with modification since the general scores are less than 50% for 3 out of the 6 AGREE II domains. As a matter of fact, we consider that it is still necessary to improve the presentations of the CPG, especially on the “Rigour of development”, “Applicability” and “Editorial independence”.

In addition, it is worth mentioning that although we used a search strategy for CPG, we did not find CPG, based of scientific evidence, including the title Paediatric Dental Emergency Management during COVID-19 pandemic. We strongly believe this is due to the recent SARS COV-2 outbreak. Consequently, we decided to include all the CPG that were related to the management of Dental Emergencies and with this, we were able to find provisional or preliminary CPG with special focus on the current context. One of these, was elaborated by the ADA though it was not prepared with the methodological rigour required for high quality CPG Table 3. Likewise, due to the little in-formation regarding our objectives in this study, we decided to include the evaluation of protocols as they play an important role on CPG elaboration.

On the other hand, it is also relevant to clarify that, only one (01) of the five (05) selected CPG was exclusively made for children (“Reference Manual for Clinical Procedures in Paediatric Dentistry” ALOP, Brazil, 2013), while two (02) of these five, were aimed at primary dentition in relation to dentoalveolar trauma (“Linee Guida Nazionali per la Pre-venzione e la Gestione Clinica dei Traumi Dentali negli individui in eta’ evolutiva”, Italy 2012) and (“International Association of Dental Trau-matology Guidelines for the Management of Traumatic dental injuries: injuries in the primary dentitition”, Sweden 2012). Likewise, “Scotland 2013” guide is aimed at management of acute dental problems. Finally, the

"Guideline for healthcare professionals" and the "Chilean CPG 2011" addressed to children and adolescents.

The CPG classification in this review showed that 17.4% of CPG are "Evidence-based", while 34.8% are based on "Expert opinion", and 47.8% are "Based on Consensus". It should be noticed that a CPG pre-pared by consensus, represents the collective opinion or suggestions of a group [17]. In contrast, a CPG, made with scientific evidence, provides recommendations from a systematic review on a specific health issue and the possible benefits or disadvantages about the different treatment op-tions [23]. Although both type of guidance documents contain sugges-tions for improving patients care and they both show their potential risks of bias [23,24]. The CPG prepared by expert opinion were excluded since their methodological quality was poor (they did not have scientific rigour), the risk of bias was high and also the conflict of interest was considerable [23].

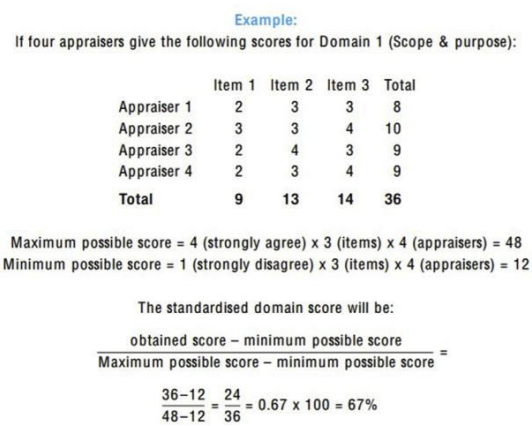

Fig. 2. Taken from: The AGREE collaboration. 2001. "Evaluation of Clinical Practice Guidelines". Appraisal of guidelines, research and evaluation: St. George's Hospital Medica School, London, June

The online training, taken by the reviewers and directed by an expert (GTR), has also been reported by other authors [34,35] and it has gained big importance for being an optimal way to guide, analyse and investi-gate through virtual platforms, during this pandemic.

4.1 About the Limits

The specific criteria for stablishing the limits of evaluation for general quality in CPG, vary widely among the different studies [36]. In our study, it was considered to assess the CPG quality in a domain-specific way, with a limit of 60% to discern whether the CPG present high, me-dium or low quality. This strategy was adopted, based on previous studies.

In this regard, Hoffmann-Eber [28], reported that global or general evaluations of CPG, using AGREE II, are not frequently performed by CPG evaluators. This study recommends making more objective evaluations by weighing individual domains of AGREE II and considering Domains III and V as key factors on the results. Based on these studies, we established 60% as the cut-off point to discern high, medium or low quality guidelines (in the global evaluation and specific evaluation by domain). In addition, it is worth mentioning that the average score for domain III "Rigour of development" should be greater than or equal to 60%, to be considered as high quality.

In a parallel analysis, if our study only adopted the global evaluation strategy, also used by O'Donnell et al. [37], the result would show that 2 CPGs (Chile, 2011 (75%) and Scotland, 2013 (50%)) would be classified as high quality CPG. In contrast to this strategy, after the spe-cific evaluation (domains) and assessment for recommendation degrees with the established limit of 60% including domain III, a more rigorous result was observed (only 1 CPG (Chile, 2011)) exhibited high quality and therefore, it was classified as recommended (R). Nevertheless, one of the main drawbacks in the application of this recommended CPG around the world, is the original language. As a matter of fact, its content is written in Spanish.

4.2 Global Quality Assessment

The evaluation of the global quality of the CPG is basically repre-sented by the average of the 6 domains. In our review, a global average score of 49.8% was found, revealing a low global average quality in all the studied CPG (Table 4), while Mubenn et al. [38] found approximate values of 51.9% (SD 13.3). Beckett et al. [39] applied the global mean score, by averaging the values of the 23 items of AGREE II and identifying the behaviour of each domain individually. In our re-view, we found that the only recommended guideline that met the criteria of the AGREE II tool, was the 2011 Chilean CPG, with an overall score of 75%; the other four CPG did not exceed 60% and therefore were considered recommended with modifications (RM), due to their low rigour of development.

Some authors question the global way of evaluating the CPG, without considering the weight of each domain. In fact, they mention that this global method is not "scientific" enough (30,33), and as a consequence, we decided to carry out the independent evaluation by domains in par-allel, to obtain a more rigorous result.

4.3 Assessment of CPG Quality by Independent Domains

Beckett et al. [39] used both forms of evaluation (Global and independent) in their study. In our study, an evaluation by independent domains was carried out, considering the importance of Domain III, similar to the studies by Jiao et al. [34] and Rabassa et al. [40].

Table 3. Quality of the 23 included guidelines on six domains using AGREE II tool

Guideline characteristics				Domains Using AGREE II									
				I	II	III	IV	V	VI	M	Domain Score		R
Title of Guideline	**Organization/ hipervínculo**	**Year**	**Country**	**Scope and Purpose (3–21)**	**Stakeholder Involvement (3–21)**	**Rigour of Development (8–56)**	**Clarity of Presentation (4–28)**	**Applicability (3–21)**	**Editorial Independence (2–14)**	**Mean Domain (SD)**	**≤30**	**≥60**	**Recomme-nded for use?**
Guidelines for dental care provision during the COVID-19 pandemic.	Available:https://www.sciencedirect.com/science/article/pii/S1013905220303266#f0005	2020	Saudi-Arabia	44	39	4	17	0	8	19% (18.6)	4	0	NR
Covid-19 Recommendations in odontology.	http://www.msal.gob.ar/images/stories/bes/graficos/0000001881cnt-covid19-recomendaciones-en-odontologia.pdf	2020	Argentina	17	11	2	17	4	0	8% (7.6)	6	0	NR
Manual de Referencia para Procedimientos Clínicos en Odontopediatría.	Available:https://www.revistaodontopediatria.org/publicaciones/manuals/referencia-para-procedimientos-en-odontopediatria-2da-edicion/Manual-de-Referencia-para-Procedimientos-en-Odontopediatria-2da-edicion.pdf	2013	Brasil	57	56	39	67	28	25	45% (17.3)	2	1	RM
Guía clínica AUGE urgencias odontológicas ambulatorias.	Available:https://www.minsal.cl/portal/url/item/7222b6448161ecb1e04001011f013f94.pdf	2011	Chile	86	88	65	85	58	69	75% (12.8)	0	5	R
Guía de práctica clínica en salud oral Infancia y adolescencia.	http://www.saludcapital.gov.co/DSP/Documentos%20Salud%20Oral/Gu%C3%ADa%20de%20Pr%C3%A1ctica%20Cl%C3%ADnica%20en%20Salud%20Oral%20Infancia-Adolescencia.pdf C:\Users\Lenovo\Downloads\Alcaldía mayor de la ciudad http:\www.saludcapital.g	2010	Colombia	72	39	15	17	4	67	36% (28.6)	3	2	NR

Guideline characteristics				**Domains Using AGREE II**									
				I	**II**	**III**	**IV**	**V**	**VI**	**M**	**Domain Score**		**R**
Title of Guideline	**Organization/ hipervínculo**	**Year**	**Country**	**Scope and Purpose (3–21)**	**Stakeholder Involvement (3–21)**	**Rigour of Development (8–56)**	**Clarity of Presentation (4–28)**	**Applicability (3–21)**	**Editorial Independence (2–14)**	**Mean Domain (SD)**	**≤30**	**≥60**	**Recommended for use?**
	ov.co\DSP\Documentos Salud Oral\Gu%C3%ADa de Pr%C3%A1ctica Cl%C3%ADnica en Salud Oral Infancia-Adolescencia.pdf												
Diagnóstico y manejo de patología pulpar y periapical.	Available:https://www.academia.edu/32275136/GPC_para_el_diagn%C3%B3stico_y_manejo_de_la_patolog%C3%ADa_pulpar_y_periapical	2016	Colombia	72	39	19	28	4	67	38% (26.9)	3	2	NR
Guía de manejo y atención en la clínica de urgencias.	http://odontologia.unicartagena.edu.co/programas-academicos/odontologia/guias-protocolos-y-manuales-de-atencion/file/10-manejo-y-atencion-clinica-urgencias	2020	Colombia	0	0	0	0	0	0	0%(0)	6	0	NR
Lineamiento técnico para la prevención y contención de COVID-19 para odontólogos y personal auxiliar de Costa Rica.	Available:https://www.ministeriodesalud.go.cr/sobre_ministerio/prensa/docs/lineamientos_odontologos_v2_27032020.pdf	2020	Costa Rica	6	0	0	11	0	8	4% (4.83)	6	0	NR
Guías prácticas de estomatología.	Available:https://www.academia.edu/36680221/Gu%C3%ADas_Pr%C3%A1cticas_de_Estomatolog%C3%Ada	2003	Cuba	56	22	6	39	63	8	32% (19)	3	1	NR
Protocolo para atención odontológica en emergencias y urgencias médicas durante la	Available:https://www.salud.gob.ec/wp-content/uploads/2020/04/PROTOCOLO-PARA-ATENCI%C3%93N-ODONTOL%C3%93GICA-EN-EMERGENCIAS-Y-URGENCIAS-	2020	Ecuador	28	28	2	39	8	25	22% (13.9)	5	0	NR

Guideline characteristics				**Domains Using AGREE II**									
				I	**II**	**III**	**IV**	**V**	**VI**	**M**	**Domain Score**		**R**
Title of Guideline	**Organization/ hipervínculo**	**Year**	**Country**	**Scope and Purpose (3–21)**	**Stakeholder Involvement (3–21)**	**Rigour of Development (8–56)**	**Clarity of Presentation (4–28)**	**Applicability (3–21)**	**Editorial Independence (2–14)**	**Mean Domain (SD)**	**≤30**	**≥60**	**Recommended for use?**
emergencia sanitaria por COVID – 19.	ODONTOL%C3%93GICAS-DURANTE-LA-EMERGENCIA-SANITARIA-POR-COVID-19.pdf												
Review of Urgent and Emergency Dental - Care in Wales.	Available:https://www.ambulance.wales.nhs.uk/assets/documents/e5e029f8-5df2-49a6-a87e-3d765beb2db4636458390559076134.pdf	2016	Gales	28	28	13	28	13	25	22% (7.5)	6	0	NR
Linee guida nazionali per la prevenzione e la gestione clínica dei traumi dentali negli individui in eta' evolutiva.	http://www.salute.gov.it/imgs/C_17_pubblicazioni_2755_allegato.pdf	2012	Italia	61	22	40	39	17	33	35% (15.6)	2	1	RM
Guía para el manejo odontológico de pacientes sospechosos o confirmados por covid-19 en las instalaciones de salud.	http://www.minsa.gob.pa/sites/default/files/publicacion-general/guia_para_el_manejo_odontologico_de_pacientes_sospechosos_o_confirmados_por_covid-19_en_las_instalaciones_de_salud_.def_.pdf	2020	Panamá	0	0	0	0	0	0	0% (0)	6	0	NR
Guía de práctica clínica: tratamiento de las enfermedades de la pulpa y de los tejidos periapicales en niños.	Ministerio de Salud - Hospital Santa Rosa http://190.102.131.45/transparencia/pdf/guiasclinicas/odonto/guia_pulpa_ninos.pdf	2017	Perú	0	0	0	0	0	0	0% (0)	6		NR
Manual de atención odontológica frente al covid-19.	ESSALUD-Red asistencial de Piura http://www.essalud.gob.pe/ietsi/pdfs/guias/Recomendaciones_Odontoesto	2020	Perú	39	17	8	17	4	0	14% (14)	5	0	NR

Guideline characteristics				**Domains Using AGREE II**									
				I	**II**	**III**	**IV**	**V**	**VI**	**M**	**Domain Score**		**R**
Title of Guideline	**Organization/ hipervínculo**	**Year**	**Country**	**Scope and Purpose (3–21)**	**Stakeholder Involvement (3–21)**	**Rigour of Development (8–56)**	**Clarity of Presentation (4–28)**	**Applicability (3–21)**	**Editorial Independence (2–14)**	**Mean Domain (SD)**	**≤30**	**≥60**	**Recommended for use?**
	matologIa_COVID.pdf												
Guía de Prácticas Clínicas estomatológicas.	Available:https://cdn.www.gob.pe/uploads/document/file/391344/Gu%C3%ADa_de_pr%C3%A1cticas_cl%C3%ADnicas_estomatol%C3%B3gicas20191017-26355-jichhz.pdf	2005	Perú	33	56	15	17	0	8	21% (20.1)	4	0	NR
Guía de Prácticas Clínicas Estomatológicas.	Hospital de apoyo NSM Carhuaz - departamento de odontología – MINSA Available:https://www.academia.edu/40984334/Gu%C3%ADa_de_Practicas_estomatolo0gicas	2019	Perú	11	0	0	0	0	0	2% (4.5)	6	0	NR
Guía de atención odontológica para COVID 19.	Colegio Odontológico del Callao Available:https://copcallao.org.pe/wp-content/uploads/2020/04/Guia-para-manejo-de-Covid-19-COP-Callao.pdf	2020	Perú	6	11	0	6	0	0	4% (4.6)	6	0	NR
Management of acute dental problems. Guidance for healthcare professionals.	Available:https://www.sdcep.org.uk/wp-content/uploads/2013/03/SDCEP+MADP+Guidance+March+2013.pdf	2013	Scotland	82	76	40	44	17	40	50% (24.6)	1	2	RM
Management of Acute Dental Problems During COVID-19 Pandemic.	Available:https://www.sdcep.org.uk/wp-content/uploads/2020/03/SDCEP-MADP-COVID-19-guide-300320.pdf	2020	Scotland	56	6	0	22	0	25	18% (21.5)	5	0	NR
International Association of Dental Traumatology guidelines for the management of traumatic dental injuries: 3.Injuries in the	Available:https://pubmed.ncbi.nlm.nih.gov/22583659/	2012	Suecia	94	67	38	39	0	25	44% (32.8)	2	2	RM

Guideline characteristics				**Domains Using AGREE II**									
				I	**II**	**III**	**IV**	**V**	**VI**	**M**	**Domain Score**		**R**
Title of Guideline	**Organization/ hipervínculo**	**Year**	**Country**	**Scope and Purpose (3–21)**	**Stakeholder Involvement (3–21)**	**Rigour of Development (8–56)**	**Clarity of Presentation (4–28)**	**Applicability (3–21)**	**Editorial Independence (2–14)**	**Mean Domain (SD)**	**≤30**	**≥60**	**Recommended for use?**
primary dentition.													
Recomendaciones del Ministerio de Salúd Pública para profesionales odontólogos e higienistas dentales. Prevención y control de coronavirus COVID-19.	Available:https://www.gub.uy/ministerio-salud-publica/sites/ministerio-salud-publica/files/documentos/noticias/MSP_RECOMENDACIONES_ODONTOLOGOS_HIGIENISTAS_DENTALES.pdf	2020	Uruguay	0	0	0	0	0	25	4% (10.2)	6	0	NR
ADA_Int_Guidance_Mgmt_Emerg-Urg_Dental_COVID19.pdf.	American Dental Association Available:https://www.ada.org/~/media/CPS/Files/COVID/ADA_Int_Guidance_Mgmt_Emerg-Urg_Dental_COVID19	2013	USA	56	11	0	0	63	8	23% (28.7)	4	1	NR
	Mean (SD)	39,3 % (30.7)	26,8% (26.6)	13,3% (18.4)	23,1% (22.4)	12,3% (20.8)	20,3% (22.4)	23.6% (25.05)			NR = 78,3%		
RM = 17,4%													
R= 4.3%													
	Median	39	22	4	17	4	8						
	Shapiro- Wilk p	0.085	0.012	<.001	0.008	<.001	<.001						

AGREE II scoring system: For each domain, scores are rated out on a 7-point scale (1 = strongly disagree, 7 = strongly agree) by individual appraisers. Individual appraiser scores are summed for an overall domain score, which is then scaled to a percentage of the maximum possible score for the domain, with higher scores indicating higher quality. The six domain scores are independent and are not aggregated into a single quality score
Overall evaluation of the guidelines according to domain score: High quality, when at least 3 domains are ≥60% (including domain III), it will be considered as Recommended (R). When the statistics are between> 30 < 60, they are determined as recommended with modifications (RM). Low quality, when at least 3 domains are ≤30 (including domain III), it will be considered Not recommended (NR)

Hoffmann-Eber [28] recommends prioritizing Domain III “Methodological rigour” and Domain II “Stakeholder involvement”. A complement to AGREE II, called AGREE REX (Recommendation of Excellence) was published in 2019. In this document, some guidelines are given on how users can classify guides as high, medium and low quality. In addition, the way in which the limits of scores can be determined by consensus among evaluators is explained, considerations about specific domains assessment are given for decision-making and limits are established based on each domain [41].

4.4 Comparison by Domains

Domain I “Scope and Purpose”: In our study, the results of Domain I reached the highest global score (76%) compared to the other domains. Likewise, it was observed that each guide presented an average score between 57% and 94%, with the highest score belonging to Sweden 2013 (94%) and followed by Chile, 2011 (86%). The other three CPGs had lower scores, due to the lack of description of the CPG scope and ob-jectives. Other authors also reported high scores in domain I [34,35,40,39].

Domain II “Stakeholder Involvement”: In our study, the results of this domain reached the second highest average score among the 5 guides evaluated with [61.8% (22%–88%)]. The CPG from Chile, 2011 (88%) and Sweden, 2013 (76%) were the highest, compared to the other 3 guidelines, which did not consider the experiences of patients and their expectations about health care. Brosseau et al. [35] and Bhatt et al. [42] found similar mean score values in this domain, in contrast [34] found a low mean score for the CPG evaluated in their study.

Domain III “Rigour of Development”: This domain is considered one of the most important and influential indicators over the quality of a guide. The results of our study revealed a low global mean score [44.4% (38– 65%)], only the Chilean CPG, 2011 showed a result of 65%, exceeding the limit of 60%. This categorised it as a “ Recommended” guide, while the other 4 CPG did not exceed the 60% limit in this domain. As a matter of fact, our results coincided with the results from other studies such as Bhatt et al. [42] and Jiao et al. [34] spe-cifically in this regard. The variability of the results is due to the lack methodological rigour, limitations description, strengths, risks, benefits, among others. A good score in this domain guarantees the quality of the guide since the risk of bias can be controlled. However, according to Downell, et al. [37], it is important to keep in mind that none of the domains are more important than the others, as they are all related to each other.

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Domain IV “Clarity of Presentation”: In our study, this domain obtained an average score of 54.8%, Chile presented 85% and Brazil 67% independently. The clarity of presentation, through key recommenda-tions, algorithms and therapeutic options that facilitate decision-making, in most of the CPG were not explicit. Clarity in the presentation was only found in the CPG of Chile and partially in the CPG of Brazil and Scotland. Other authors [34,35,42], reported a global score greater than 60% in this domain.

Domain V “Applicability”: The lowest global score in our review was observed in this domain (24%). The CPG of Chile obtained 58% and the others showed scores lower than 28%. Similar and even lower results were reported by Broseau et al. [35], and Jiao et al. [34] whose studies showed 14% and 31.25%, respectively. While Bhatt et al. [42] showed values of 48% (10–96) and Beckett et al. [39] 54.8% (20.1). These results indicate that the majority of the CPGs did not present information on the facilitators, barriers and financial re-sources for the application of the recommendation; they only described the intention to carry it out without providing an implementation strat-egy. It is important that the CPG have an understandable format, including graphs and algorithms for decision-making. Regarding this, in our review, the Scottish CPG, 2013, presented an interactive electronic decision support tool, based on the information contained in this guide (http://madp.sdcep.org.uk/).

Domain VI “Editorial Independence”: Conflict of interest and Editorial independence have not been reported in detail in the majority of the CPG evaluated in this review. The average score that was obtained was 38.4%, independently. The Chilean CPG presented 69%, being different from the rest of the evaluated CPG. Other authors [37,35,42] also reported this domain as the lowest in their reviews. As a matter of fact, financial institutions seldom make an explicit declaration that their views or interests have not influenced the final recommendations. Re-ports of conflicts of interest and participation of financial entities in the development of guidelines are crucial for the assessment of this domain [37,34].

In other aspects, Burgers JS [43] conducted a comparison study be-tween North American and European CPG and found that European CPG exhibited a better quality. In our systematic review, the best quality CPG is from South America, Chile, 2011. This is due to the scientific progress this country has had in recent years and the large investment in public health development. Although Burgers JS mentioned that most of the high-quality guides have been developed by organizations in countries with more resources and funds for research (e.g. United Kingdom, United States, Canada etc.); in our review, we were able to verify that there are good quality CPG in developing countries, such as Chile, Mexico and Brazil. These countries present attractive proposals that could be modi-fied and translated into the universal language for their application worldwide.

Table 4. Quality of the 5 dental guidelines report by domain

Guideline characteristics			**Domains using AGREE II**									
			I	**II**	**III**	**IV**	**V**	**VI**	**Global Average**	**Domain score**		**Recommendations**
Title of Guideline	**Year**	**Country**	**Scope and Purpose**	**Stakeholder Involvement**	**Rigour of Development**	**Clarity of Presentation**	**Applicability**	**Editorial Independence**	**Mean (SD)**	**≤30**	**≥60**	
Guía clínica AUGE urgencias odontológicas ambulatorias	2011	Chile	86	88	65	85	58	69	75% (12.8)	0	5	R
Management of acute dental problems. Guidance for healthcare professionals	2013	Scotland	82	76	40	44	17	40	50% (24.6)	1	2	RM
Manual de Referencia para Procedimientos Clínicos en Odontopediatría	2013	Brasil	57	56	39	67	28	25	45% (17.2)	2	1	RM
International Association of Dental Traumatology guidelines for the management of traumatic dental injuries: 3.Injuries in the primary dentition	2012	Suecia	94	67	38	39	0	25	44% (32.8)	2	2	RM
Linee guida nazionali per la prevenzione e la gestione clínica dei traumi dentali negli individui in eta' evolutiva	2012	Italia	61	22	40	39	17	33	35% (15.6)	2	1	RM
	Mean (SD)	76.0 (16.2)	61.8 (25.2)	44.4 (11.5)	54.8 (20.5)	24.0 (21.5)	38.4 (18.2)	49.8 (19.6)	1.4	2.2		
	Median	82	40	67	44	17	33					
	Shapiro- Wilk p	0.392	0.001	0.639	0.142	0.528	0.104					
Evaluation of the Global quality of the CPG according to the Domain score												
High quality	3 domains> = 60% including domain III	Recommended (R)										
Medium quality	> 30 < 60	Recommended with modifications (RM)										
Low quality	3 domains ≤ 30% including domain III	Not Recommended (NR)										

Overall evaluation of the guidelines according to domain score: High quality, when at least 3 domains are 60% (including domain III), it will be considered as Recommended (R). When the statistics are between> 30 < 60, they are determined as recommended with modifications (RM). Low quality, when at least 3 domains are 30 (including domain III), it will be considered Not recommended (NR)

4.5 Implication in the Development of New Guidelines

The present lack of rigour in the development of CPG on dental emergencies, encourages us to develop new CPG based on high quality scientific evidence, to generate grades of recommendation aimed at the paediatric population.

The institutions in charge of elaborating CPG require a team of ex-perts, internal and external, for the development of guides, complying with the methodological rigour.

A short-term measure is to update the high-quality CPG available and associated with the research topic of the guide to be developed. For this purpose, it is necessary to strengthen the cooperation of methodological experts, seek patients (opinions from the public) to improve the appli-cability of the CPG, solve financing problems and define conflicts of in-terest in a clear way.

4.6 Strengths and Limitations

The strategy used for searching CPG constitutes one of the strengths of the present study. A meticulous investigation in the different guideline compilers and governmental entities from different countries, was car-ried out. The manual search of CPG applicable to the current context of the COVID 19 pandemic and the gray literature, provided additional value for obtaining eligible guidelines.

The world is undergoing a dreadful pandemic and this current context forces us to seek and provide quick solutions. The development of new knowledge on CPG is necessary. The management of emergencies in this context is relevant and so are high quality guidelines.

Nevertheless, this COVID-19 pandemic also represents one of the limitations, since in this context, the administrative processes that favour the adequate preparation of high-quality CPG for the management of paediatric dental emergencies are slowed down. In addition, specific CPG regarding this topic and written in the international language (English) are not available. As a matter of fact, this aspect represents a great lim-itation for our review.

4.7 Recommendations for Future Studies

For future research, it would be interesting to study the relationship between the quality of the guidelines and the effectiveness of the guidelines' recommendations in different countries because the eco-nomic, social and cultural realities of each country are different.

5. CONCLUSIONS

High, medium and low quality CPG for the management of paediatric dental emergencies were found. It is necessary to pay especial attention to the AGREE

II domains so as to improve the CPG quality and apply them during the COVID-19 pandemic.

According to the quality evaluation criteria and recommendation degree of the AGREEII instrument, only one CPG (AUGE clinical guide for ambulatory dental emergencies- Chile, 2011) was considered a “Rec-ommended” CPG, but applicable only among Spanish-speaking coun-tries. It would be advisable to work on this guide, using English as an international language.

AUTHOR CONTRIBUTION

All authors listed have significantly contributed to the development and the writing of this article.

FUNDING STATEMENT

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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SUPPLEMENTARY MATERIALS

S1. (PRISMA) Checklist of Items to include when a systematic review or meta-analysis is published

Section	Item	Prisma checklist item	Reported on Page #
Title			
Title	1	Identify the publication as a systematic review, meta-analysis, or both.	Page 1, li 1-3
Abstract			
Structured summary	2	Provide a structured summary that includes, as appropriate: background; objectives; data source; eligibility criteria for studies, participants and interventions; evaluation of the studies and synthesis methods; results; limitations; conclusions and implications of the main findings; registration number of the systematic review.	Page 1, li 5 - 38
Introduction			
Rationale	3	Describe the rationale for the review in the context of what is already known about the topic.	Page 2, li 71
Objectives	4	Explicitly state the questions to be answered in relation to participants, interventions, comparisons, results, and study design (PICOS).	Page 2, li 87
Methods			
Protocol and registration	5	Indicate if there is a review protocol that can be accessed (for example, web address) and, if available, registration information, including your registration number.	Page 3, li 92
Eligibility criteria	6	Specify the characteristics of the studies (for example: PICOS, duration of follow-up) and of the characteristics (for example: years covered, languages or publication status) used as eligibility criteria and their justification.	Page 3, li 102
Information sources*	7	Describe all information sources in the search (for example, databases with dates of coverage and contact with authors to identify additional sources), as well as the date the most recent search was executed.	Page 4, li 120
Search	8	Present the full electronic search strategy for at least 1 database, including any limits used, such that it could be repeated.	Page 4, li 121
Selection of sources of evidence†	9	State the process for selecting sources of evidence (i.e., screening and eligibility) included in the systematic review and, where relevant, included in the meta-analysis	Page 4
Data charting process‡	10	Describe the methods of charting data from the included sources of evidence (e.g., calibrated forms or forms that have been tested by the team before their use, and whether data charting was	Page 4

Section	Item	Prisma checklist item	Reported on Page #
		done independently or in duplicate) and any processes for obtaining and confirming data from investigators.	
Data items	11	List and define all variables for which data was searched (for example, PICOS source funding) and any assumptions and simplifications that have been made.	Page 4
Risk of bias in the studies individual	12	Describe the methods used to assess risk of bias in individual studies (specify whether it was done at the study or outcome level) and how this information is used in data synthesis	Page 5
Summary measures	13	Specify the main summary measures (for example: risk ratio or difference in socks).	Page 6
Synthesis of results	14	Describe methods for handling data and combining study results, if any, including measures of consistency (for example, quantification of heterogeneity by the statistical index I2) for each meta-analysis	Page 6
Risk of bias between studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (for example, publication bias or selective communication).	Page 6
Additional analysis	16	Describe additional methods of analysis (for example, sensitivity or subgroup analysis, meta-regression), if any, indicate which ones were pre-specified.	-
Results			
Study selection	17	Give numbers of sources of evidence screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally using a flow diagram.	Page 6
Characteristics of sources of evidence	18	For each study, present the characteristics for which the data were extracted (for example: size, PICOS, and length of follow-up) and provide bibliographic citations.	Page 6
Risk of bias in the studies	19	Present data on the risk of bias in each study and, if available, any assessment of bias in the results (see item 12)	Page 6
Study results individual	20	For each result considered for each study (benefits or harms), present: a) the data summary for each intervention group and b) estimate of the effect with its confidence interval, ideally graphically using a forest plot.	Page 6
Synthesis of results	21	Present results of all meta-analyzes performed, including confidence intervals and consistency measures.	Page 6
Risk of bias between studies	22	Present the results of any assessment of risk of bias between studies (see item 15)	Page 6
Additional analysis	23	Provide the results of any additional analyzes, if they have been performed (for example, sensitivity or subgroup	-

Section	Item	Prisma checklist item	Reported on Page #
		analysis, meta-regression (see item 16)).	
Discussion			
Summary of evidence	24	Summarize the main findings, including the strength of the evidence for each outcome principal; consider their relevance to key groups (for example: caregivers, users and decision-makers in health).	Page 7
Limitations	25	Discuss limitations of studies and results (e.g. risk of bias) and of the review (for example: incomplete collection of identified studies or communication selective).	Page 7-10
Conclusions	26	Provide a general interpretation of the results in the context of other evidence as well as the implications for future research.	Page 11
Funding			
Funding	27	Describe the sources of funding for the systematic review and other support (for example, data input), as well as the role of funders in the systematic review.	Additional page

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This chapter is an extended version of the article published by the same author(s) in the following journal.
Heliyon, 6 (e05612), 2020.

Chapter 10
Print ISBN: 978-93-5547-742-2, eBook ISBN: 978-93-5547-749-1

Determining the Correlation between Types of Thyroid Surgery, Goiter Pathology and Recurrent Laryngeal Nerve Injury

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DOI: 10.9734/bpi/cpms/v9/2966A

ABSTRACT

Background: Thyroidectomies are often performed surgical procedures that entail the partial or complete removal of the gland depending on the nature and pathology of the goitre. The Recurrent Laryngeal Nerve Injury (RLNI) represents nearly half of all the complications of thyroid surgery. Vocal cord paresis or paralysis, due to iatrogenic injury of the recurrent laryngeal nerve, is one of the main problems in thyroid surgery. Although many procedures have been introduced to prevent the nerve injury, still the incidence of recurrent laryngeal nerve palsy varies between 1.5-14%. The aim of this study is to Estimate the recurrent laryngeal nerve palsy concerning different types and indications of thyroid surgery. A cohort retrospective study of 705 patients with different kinds of goiters admitted to Al-Sadder teaching hospital and Al-Ameer private hospital in Najaf city- Iraq, for thyroid surgery. The current study is based on a single surgeon's experience from 1 October 2007 to 30 June 2018, with a mean follow-up time of 37 months. Seven hundred five patients underwent various thyroidectomies for various purposes. The majority of patients have benign multinodular goitres (67.4 percent). Indications for surgery for toxic multinodular goitres, which have been observed in (19.15 percent). Malignancy was observed in (14.3%), while the least frequent reasons for surgery in our sample were Hashimoto's thyroiditis and Grave's disease (7.6%% and 3.4%), respectively. Temporary recurrent laryngeal nerve injuries have been seen in (0.69%) of subtotal surgery rising to (10%) (p=0.05), for patients having recurrent goitres. The overall permanent recurrent laryngeal nerve injuries were (0.99%) ranging from (0%) in subtotal up to (5%) (p=0.027) in cases for recurrent goitres. Total thyroidectomy is preferred since it will lessen the need for repeat surgery, which is accompanied by a considerably higher rate of nerve injury, even though partial thyroidectomy is associated with a low rate of recurrent laryngeal nerve injury.

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Keywords: Thyroidectomy; post-operative recurrent laryngeal nerve injury; temporary; permanent; vocal cord injuries.

1. INTRODUCTION

Theodor Kocher was the first surgeon to perform a comprehensive thyroidectomy in 1909 with a low risk of recurrent parathyroid and laryngeal nerves. His success caused surgical mortality to drop from 50% to under 4.5% as a result of his work [1]. In the past 25 years, complete thyroidectomy has taken the position of bilateral partial thyroidectomy as the treatment of choice for all Graves' disease patients, bilateral benign multinodular goitres, and all patients with thyroid cancer other than those with very low risk [2]. The complication rate after thyroid surgery varies widely from surgeon to surgeon and from center to center. The Recurrent Laryngeal Nerve Injury (RLNI) represents nearly half of all the complications of thyroid surgery [3-5]. The RLNI after thyroidectomy, although infrequently encountered, can jeopardize the quality of life [6]. In addition to the hoarseness that occurs with unilateral RLN Injury, bilateral RLN Injury leads to dyspnea and often life- threatening glottal obstruction [7,8]. The incidence of RLN injury is higher during re-exploration, Graves' disease, thyroid carcinoma procedures and non-identification of RLN during surgery. However, there was no significant difference in the incidence of recurrent laryngeal nerve injury with regards to gender [9-11]. Surgically induced recurrent laryngeal nerve paralyzes are frequently not recognized at the time of thyroid surgery. The exact incidence of recurrent laryngeal nerve injury is still controversial [12]. The incidence of Injuries to the recurrent laryngeal nerve has been reported between 1% to 2% from different thyroid surgery centers when performed by experienced neck surgeons. Age, retrosternal goitre, routine laryngoscopy, re-operation, nodal Dissection, bilateral thyroidectomy, RLN monitoring and surgeon volume were significantly associated with RLN palsy. Post-operative haematoma showed no significant correlation to surgeon volume. Categorisation of annual rate of surgeon's volume (AR) showed that RLN palsy rates declined in surgeons performing >50 cases/year to a minimum of 3% and 2.6% respectively in highest volume AR group (>100 cases/year) [13]. Although many procedures have been introduced to prevent the nerve injury, still the incidence higher when performed by less experienced surgeons to reach between 1.5-14% [11,14,15]. Appropriate understanding of the emberyology & anatomy of the RLN, surgical experience, attention to surgical details at certain site and the use of intraoperative nerve monitoring (IONM), especially in bilateral operations & in malignant cases make completion of surgery more safe [16,17]. RLN nerve was not identified routinely. The operating surgeon checked vocal cord mobility at the time of extubation. Postoperatively, indirect laryngoscopy can be done on the second and fifteenth postoperative day (when laryngeal edema subsides), and repeated as required later on [18]. Despite no apparent surgical insult, up to 2% of patients may have RLN, vocal cord paralysis without any recognized intra-operative event [19]. Up to 50% of patients with paralysis of their vocal cords may be asymptomatic (subclinical) [20,21]. Echternach et al (2009) conclude that laryngeal complications after thyroidectomies are primarily caused by injury to the vocal folds from intubation and to a lesser extent by injury to the laryngeal nerve [22].

Mechanisms of injury to the nerve include complete or partial transection, traction, or handling of the nerve, contusion, crush, burn, clamping, misplaced ligature, and compromised blood supply [23,24]. Dysphonia starting on the 2nd – 5th postoperative days is commonly due to edema, whereas traction injury of the nerve and damage of axons may result in dysphonia lasting up to 6 months. Dysphonia continuing after 6 months is commonly permanently caused by cutting, ligating or cauterization of the nerve [25]. Bilateral RLNI is much more serious, because both vocal cords may assume a median or paramedian position and cause airway obstruction and tracheostomy may be required. Accidental transaction commonly occurs at the level of the upper two tracheal rings, where the nerve closely approximates the thyroid lobe in the area of Berry's ligament [26,27].

2. MATERIALS AND METHODS

A cohort retrospective study involving 705 patients undergoing different thyroid surgical procedures, the current study sheds light on an experience of one surgeon, (including radical neck dissection in indicated cases) for different indications, from October 2007 to June 2018 in Al-Sadder medical city teaching hospital, Al-Ameer private hospital in Al-Najaf governorate / Iraq. Data includes recording proper history and clinical examination with general and specific investigations (thyroid function test, ultrasound of the neck), and fine-needle aspiration cytology was done to the patients in indicated cases. Preoperatively, patients send for otolaryngologists for vocal cord exams. Patient's sign on the consent for type of thyroid operation under general anesthesia have been done and classical operations (Total thyroidectomy TT = bilateral total lobectomy and isthmusectomy, Subtotal thyroidectomy STT = bilateral subtotal lobectomy leaving 4gm of normal thyroid tissue on each side and isthmusectomy, Near-total thyroidectomy NTT= total lobectomy, isthmusectomy, and subtotal lobectomy) have been carried out under general anesthesia and suitable positioning. Hemostasis achieved without use of any non- absorbable suture material in most of cases, while surgical clips and harmonic cauterization used in few cases and recording operative and post-operative course. Follow up was done for 6-120 months with a mean period of 37 months as outpatient visits. Vocal cords were examined by the anesthetist at time of extubation and at 6 weeks, 3 & 6 months later by otolaryngologist, some cases have been re-examined 12 months post-operatively in indicated cases. The collected data has been analyzed and compared with the other studies. Chi-square and z-test had been applied to test the similarities and differences between categorical variables which are considered significant at the level of $\alpha \leq 0.05$. This work has been reported in line with the STROCSS criteria [28].

3. RESULTS

Seven hundreds and five patients were included in the present study, underwent different types of thyroid surgery for different types of thyroid pathologies. Most of patients 615 (87.2 %) were female with male to female ratio (1:6.8). Patient's ages ranged from 11 – 80 years. Most of them aged from 20- 60 years (607 patients

86.1%), with a peak incidence in the 4th & 5th decades of life, there were 475 patients (67.4%) with non-toxic multinodular goiter (non-TMNG). The second commonest pathology was toxic multinodular goiter (TMNG) occurring in 135 patients (19.15%). Malignant thyroid tumor was seen in 101 patients (14.3%), followed by Hashimoto's thyroiditis in 54 patients (7.6%), graves' disease in 24 patients (3.4%), while the least incidence was seen with simple goiter in 17 patients (2.4 %), as shown in Table (1).

Female gender was involved more than male in a different type of pathologies, but there was a significant difference with p. value (<0,012) regarding the occurrence of Hashimoto's thyroiditis in female patients. There was a significant difference concerning the occurrence of malignancy in male patients 48.9 % (44/90) in comparison to female patients 9.3 % (57/615), P-value <0.001. Also a significant difference in males affected by TMNG, P-value = 0.026. As shown in Table (2). There was no significant difference regarding affection of both genders by non-TMNG, Grave's disease & simple goiter, P-value were 0.693, 0.561 and 0.9 respectively. STT done for 145 patients (20.5%) in the early face of the study while NTT & TT were done for 560 patients (79.5%) mostly in the last eight years of the study. TT and radical neck dissections have been carried out for 31 patients (4.4 %) having a preoperative diagnosis of thyroid carcinoma. Thyroid with unilateral or bilateral lymph node metastasis and for one case (0.32 %) having tuberculous lymphadenopathy + MNG. Forty patients (5.67 %) had operations for recurrent goiters of different pathologies.

RLNI was significantly low in STT and NTT in comparison with TT & operations for recurrent goiters, (P value= 0.001). Temporary RLNI was significantly low in goiters treated by STT (0.69%) as compared with (4.97%) and (10%) of goiters managed by TT and in operations for recurrent goiters respectively, (p value=0.05). The same was true regarding Permanent RLNI that seen in our patients treated by STT and NTT with those who were treated by TT as a first-time surgery or for recurrent goiters with the highly significant difference rate (p value= 0.027), as shown in Table (3).

Our study shows a non-significant difference regarding temporary RLN and permanent RLN injuries among patients having operations for different histomorphological types of goiters, as in Table (4). All the RLNI were unilateral and no need for tracheostomy for any patient included in this study.

During the follow up of our patients in the present study, we noticed that complete recovery of most of the temporary RLN injuries happened during the first 3 months after surgery (77.4%). More rapid recovery seen during the first 6 weeks postoperatively in (22.6%). While the remaining patients recovered after 6 months or more as in Table (5).

Table 1. Distribution of patients with thyroid diseases by age group and gender

Age group year	Non-TMNG		Hashimoto's thyroiditis		Graves' disease		Total MNG		Malignancy		Simple goitre		Total	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F
11 – 20	2	8	0	8	0	2	0	6	0	4	0	2	2	30
21- 30	7	72	1	21	0	3	2	32	10	11	0	7	19	147
31- 40	25	130	0	11	4	6	17	30	13	13	1	6	60	196
41– 50	16	116	0	8	0	8	2	28	7	13	1	0	26	173
51– 60	7	66	0	5	0	1	4	12	14	7	0	0	25	91
61– 70	2	19	0	0	0	0	0	2	0	7	0	0	2	28
71– 80	0	5	0	0	0	0	0	0	0	2	0	0	0	7
Total	59	416	1	53	4	20	25	110	44	57	2	15	90	615
%	12.4	87.6	1.9	98.1	16.7	83.3	18.5	81.5	43.6	56.4	11.8	88.2	12.8	87.2
Total	475		54		24		135		101		17		705	

Table 2. Distribution of different types of goiters according to gender

Type of goiter		**Hashimoto's disease**	**Other diseases**	**OR (95%CI)**	**P value**
Gender	Female	53	562	8.39 (1.14-	0.012
	Male	1	89	61.4)	
Type of goiter		Malignancy	Other diseases		
Gender	Female	57	558	9.36	< 0.001
	Male	44	46	(5.7-15.3)	
Type of goiter		Non-TMNG	Other diseases		
Gender	Female	416	199	0.91	0.693
	Male	59	31	(0.57-1.45)	
Type of goiter		Grave's disease	Other diseases		
Gender	Female	20	595	1.38	0.561
	Male	4	86	(0.46-4.14)	
Type of goiter		Toxic MNG	Other diseases		
Gender	Female	110	505	1.76	0.026
	Male	25	65	(1.06-2.92)	
Type of goiter		Simple goiter	Other diseases		
Gender	Female	15	600	0.909	0.9
	Male	2	88	(0.204-4.04)	

Table 3. The post-operative RLN injury were recorded and classified according to the type of surgery

Complication	STT n=145	NTT n=137	TT n= 423	Op for recurrent goiter n= 40	TT + radical neck dissection n= 31	Total n= 705	P value
Temporary RLNI	1	4	21	4	1	31	0.05
Permanent RLNI	0	1	3	2	1	7	0.027
Total	1	5	24	6	2	38	0.001

Table 4. Distribution of the post-operative RLN injury in the various types of histopathology

Histopathology	Non TMNG N=475	TMNG N=135	Simple N=17	Grave's disease n=24	Hashimoto's thyroiditis n=54	Malignancy n=101	Total n=705	P value
Temporary RLNI	13	8	0	2	1	7	31	0.139
Permanent RLNI	6	0	0	0	1	0	7	0.565
Total	19	8	0	2	2	7	38	0.586

Table 5. The relation between time of recovery from temporary RLNI and type of surgery

Time	STT	NTT	TT	TT+ radical neck dissection	Re- operative surgery	total
3 months	1	2	18	1	2	24
6 months		2	3		2	7
Total	1	4	21	1	4	31

4. DISCUSSION

Patients between 21-60 years old were the most common age group affected in the present study, with an overall mean age incidence of 39± 14.5 years. This finding will not correspond to Leigh Delbridge et al. [29] study where the mean age was 53±14. Most of our patients were females with (M: F) ratio of (1:6.8), which is less than Antonio Rois et al. [30] study, who found (M: F) ratio of (1:11) and comparable to (1:7) of Leigh Delbridge et al. study [29] 1:6.2 of Iyomasa RM et al study [31] and higher than 1:3.25 ratio seen from Hazem M. Zakaria et al study [11]. The current study shows the transient and permanent RLN palsy in (4.4%) and (0.99%) respectively. These findings are compared to many other studies as seen in the following Table (6). This complication is generally unilateral and transient, but occasionally it can be bilateral and permanent and it may be either deliberate or accidental [18,32]. The permanent lesion of damaged RLN often manifests as an irreversible dysfunction of phonation. It is the most common complication following thyroid surgery [33]. TT & NTT was done for 560 patients, including premium and those with first and second-time recurrence goiter of different clinical and histological presentation, most of them have a smooth post-operative follow up except for 30 patients (5.36 %) who had temporary RLN injury while permanent RLN injury developed in 7 cases (1.25 %). This finding was comparable with Hazem et al. [11] findings which were (6.9% & 0.72%) and less than Chaudhar et al. [18] findings which were (7.69 % & 3.84%) for temporary RLN injury & permanent RLN injury respectively. Our findings were much less than Aytac et al. [32]. Kasemsuwan et al. [10] and Iyomasa RM et al. [31], our results were higher than Jensen PV et al [33] who had their results for surgery on benign thyroid conditions only, Table (6).

The similarity and differences seen in the above-mentioned studies' findings can be explained by multifactorial reasons like the size of the study, the type of surgery (premium or redo, unilateral or bilateral), type of histopathology and the experience of the surgeons. Subtotal thyroidectomy has been achieved in 145 patients with (0.69 %) TRLN and no permanent injury had been recorded. This finding was less than Chaudhary et al. 15 finding which were (1.53%) and with Hazem et al. [11] finding which were (1.9%) for temporary injury and no permanent injury. We illustrate the comparison in the result between different studies and our study regarding TT and STT as in Table (7). The incidence of the RLN injury in redo operations is (15 %) while in primary operations is (4.81 %), p value=0.001. This finding is comparable with Hazem et al.11 which was (21.7% in redo vs. 4.1% in primary, p=0.001), Pantvaidya G et al. [34] findings (16.2 % vs 9 %) for redo & primary operation, respectively, and to Landerholm K, et al. [35], Dhillon VK et al who state an increased risk with redo surgery, malignant disease and total thyroidectomy operations. The incidence of nerve injury in malignant disease was 6.93 % in present study, which is comparable to Landerholm K et al [36] study with (5.9 %) and much less than Hazem et al. [11] with 12.8 % in malignant conditions, and much lower than Iyomasa RM et al. [31] study that reports (31.3 %).

Table 6. Comparison between our study and other studies regarding the incidence of Temporary RLNI & permanent RLNI

Study	Year of study	Number of patients	TRLNI %	PRLNI %
Present study	2018	705	4.4	0.99
Chaudhar et al. [18]	2007	310	2.58	0.64
Hazem M. Zakaria et al [11]	2011	340	4.1	0.3
Pantvaidya G et al. [34]	2018	152	11.2	3.9
Dhillon VK [37]	2018	1547	2.9	0.4
Iyomasa RM et al. [31]	2017	151	22.5	6.6
Jensen PV et al. [33]	2015	114	1.8	0.9
Landerholm K et al. [35]	2014	64699	3.52	1.2

Table 7. Comparison between different studies and our study regarding TT, STT and the incidence of temporary RLNI and permanent RLNI

Author	Year	Procedure	No. of patients	TLRNP %	PRLNP %
Present study	2018	STT	145	0.69	0
		TT & NTT	560	5.36	1.25
Landerholm K et al [35]	2014	TT	64699	2.74	0.75
Chaudhary et al. [18]	2007	STT	310	1.53	0
		TT		7.69	3.84
Aytac et al. [32[	2005	TT	418	13.6	9
Kasemsuwan et al. [10]	1997	TT	105	6.7	7.6
Hazem et al. [11]	2011	STT	340	1.9	0
		TT & NTT		7.2	0.94
Iyomasa RM et al. [31]	2017	TT	151	22.5	6.6
Pantvaidya G et al. [34]	2018	TT	1547	2.9	0.4
Jensen PV et al [33]	2015	TT	114	1.8	0.9

Most of our patients 77.4 % were recovered from dysphonation symptoms within 6-12 weeks post RLN operatively while it needs 6-9 months in the study of Pantvaidya G et al. [34]. This finding can be explained by the little insult that the exposed to (traction or contusion injury) in our patients in whom we avoid diathermy during dissection of the thyroid gland, although the most effective method for protection of RLN from injury is still controversial. Some surgeons claim that omitting the identification of RLN may cause little trauma. However, other studies have proved that this is not true [36,37]. Opposing the first idea, we agreed that the identification of RLN during operation requires the surgeon to know of the anatomic course of the nerve and its' variations lead to decreased RLNI incidence [38,39]. Still, good intra-operative hemostasis and the use of loupe magnification are essential for nerve identification and preservation [40,41].

5. CONCLUSION

Although subtotal thyroidectomy accompanied by a low rate of recurrent laryngeal nerve injury, still total thyroid removal is preferable because it will prevent the need for redo surgery, which associated with a significantly higher rate of recurrent laryngeal nerve injuries, and will guarantee removal of carcinomatous tissue.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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DISCLAIMER
This chapter is an extended version of the article published by the same author(s) in the following journal.
Journal of Surgery and Research, 3 (2): 086-095, 2020.

Chapter 11
Print ISBN: 978-93-5547-742-2, eBook ISBN: 978-93-5547-749-1

Proteomics in Human Healthcare: A Tool in Disease Diagnosis

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DOI: 10.9734/bpi/cpms/v9/2734A

ABSTRACT

Proteins are the most relevant markers of cell functions as they represent the functional output of a cell. Systematic studies of the human proteome from both body fluids and tissue samples may be a dynamic and powerful approach towards better understanding numerous malignancies where traditional methods of diagnosis may not be enough. Alterations in the protein profiles may be detected in cells in their normal non-diseased states. Further comparing them with that of diseased state can extend the base information regarding the underlying pathogenesis of a disease. Proteomics has been making giant strides towards uncovering basic biological processes and aiding in better knowhow of some complex biological phenomena involved in some major diseases. This chapter will focus on such studies where various body fluids and tissue samples have been put to several proteomic studies to understand the pathologies of some diseases. Body fluids such as plasma, urine, sputum, CSF, seminal fluid, cervicovaginal fluid, etc., as well as various tissues such as tissues from brain, breast, liver, ovary, vagina, etc., have been used by researchers to study diseases associated with these tissues. Furthermore, continuous advancements and refinements in proteomic techniques will usher healthcare into a revolutionary era of early diagnosis, prognosis and ultimately lead to successful treatment of these human malignancies.

Keywords: Human healthcare; human proteome; biological processes.

1. INTRODUCTION

With the ever-increasing burden of diseases due to dynamically changing lifestyles and habits of the global population, the need for more innovative and less invasive diagnosis becomes even more critical. The diagnosis technique through biological samples using proteomic studies has given significant hope to researchers worldwide to work in this direction. Proteomics has emerged as a powerful tool in the field of biomedical research. It involves analysing the protein

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component of a cell, organ tissue, or biological fluids under certain conditions that may be its natural state or diseased condition. Additionally, it is being applied to study the proteins of a cell or tissue after induced laboratory conditions. Protein analysis has become a reliable technology providing us a unique insight into cell physiology and its functions and broadening our understanding of body functions. The Study of proteins proves to be most relevant when we investigate the differentially expressed proteins in a normal state versus the diseased condition of the cell/tissue or fluids and can be used in the diagnosis and treatment of diseases. Also, it can be used in the early detection of diseases [1,2].

Furthermore, the differential expression of proteins is either the cause or the effect of physiological changes in the cell. Identifying these changing states of proteins will characterize their biological roles, providing us further clarification of some biological mechanisms. It will ultimately lead to the identification of therapeutic targets and diagnostic biomarkers [3,4].

Why study proteins? The answer lies in the fact that proteins play a crucial role in body functions by controlling biosynthesis, maintaining cell communications, proliferation, metabolism, immune functions, and also provides structural stability to tissues/organs in living beings [5]. Proteins can be termed as the most relevant markers of cell functions because they represent the functional output. They encompass all the changes occurring over time depending on changes in the cell microenvironment and describe the actual cell conditions as they end with biological functions. Although proteins are a product of gene expressions after translation of mRNA, posttranslational modifications can alter and ultimately determine the protein's role in the cell. Study of proteins is more advantageous as compared to direct mRNA expression studies because of various reason as firstly, proteins, not mRNA, are the functional molecules for most relevant physiological pathways; Secondly, though, changes in mRNA abundance can affect the change in quantities of proteins, but it is not the only mechanism which causes this change [6,7]; Thirdly, posttranslational changes such as glycosylation, phosphorylation, and proteolytic maturation can lead to modifications in the structure and function of the proteins and these can only be detected by directly studying the proteins involved. Therefore, it becomes even more essential to study the proteins rather than mRNA to assess the actual changes in the cell microenvironment [8,9]. Thus, proteomic studies can help us identify, quantify, and characterize many or all proteins that play a role in a particular pathway of cell, tissue, or organ. These proteomic studies could be performed on both body fluids or tissues as proteins from body fluids such as blood, urine, or vaginal tract fluid give us great clarity into the changes undergoing in specific tissues or overall, in the body and by studying the tissue proteomics, we will also know about the proteins that are present in the intact tissue as some proteins are not normally secreted from the cells unless cell lysis occurs [10,11]. So, considering all these factors, it is safe to say that proteins reflect the actual status of a cell, and the proteome of the cell is dynamic and changes according to the cell's physiological conditions. Thus, studying the differential protein expression can act as a reporter to define healthy cells from

diseased states and hence, as a potential source in early detection and diagnosis of various diseases.

2. PROTEIN STUDIES ON VARIOUS BIOLOGICAL FLUIDS

Biological fluids are the prime targets for proteomic analysis owing to low invasiveness, minimum cost, and accessible sample collection and processing. The body fluids such as plasma/serum, urine, sputum, cerebrospinal Fluid, amniotic Fluid, seminal Fluid, cervicovaginal fluid/vaginal fluids, etc., provide us with a rich source of proteins and molecules which are significantly altered in response to different biological states of the body [12].

2.1 Plasma/Serum

Blood plasma/serum is believed to have the highest amount of proteins among the body's fluids. Because of its access to almost every part of the body, it contains various proteins and molecules secreted into it by multiple organs [13]. This has attracted the interest of multiple scientists, and numerous studies have been performed on its proteome, and till today, over 12,000 different proteins have been identified in it. This provides a large set of circulating proteins to be used as potential biomarkers in clinical diagnosis such as cardiovascular diseases, ovarian cancer, among numerous others.

Cardiovascular diseases (CVD) are the leading cause of death for 30% of deaths worldwide. Domanski *et al.,* 2012 performed proteomic analysis on the blood plasma of several patients suffering from CVD and listed 67 proteins as biomarkers having roles ranging from coagulation and thrombolysis pathway, inflammatory markers, several acute phase reactants, and several lipoprotein components [14].

Ovarian cancer is the most lethal gynecological malignancy affecting women today. The disease is often diagnosed in its later stages and is often difficult to control by then. Li *et al.,* 2012 used blood serum from patients of malignant ovarian tumor (MT), benign ovarian tumor (BT), and healthy donors as controls [15]. They identified several proteins which were different among these three groups, including 124 proteins that showed differential expression between the MT and BT groups. Among these, two proteins, i.e., APOA4 (Apolipoprotein A-4) and NRAMP1 (natural resistance-associated macrophage-1) were described as potential biomarkers for the malignant ovarian tumor in women with elevated serum CA125 (Cancer antigen 125).

2.2 Urine

Urine is a complex fluid and contains various proteins from the blood and also from the kidneys and urogenital tract as it passes out through them. To date, around 8000 proteins have been identified in it [16] and can be used in various urogenital diseases such as bladder cancer and diabetic nephropathy.

Bladder cancer is more frequently observed in men, and as the case with all cancers, early diagnosis can significantly help in its successful treatment. Orenes *et al.,* 2007 obtained urinary samples from patients presenting haematuria suspected of having bladder cancer and healthy donors as control [17]. Proteomic analysis showed the increased expression of some proteins between the bladder cancer patients and controls. These were identified as Reg-1 (Regenerating protein) and keratin-10. Further, immunohistochemistry procedures on bladder cancer tumors of the same patients confirmed the presence of Reg-1 in them, thus identifying Reg-1 as a biomarker in the diagnosis of bladder cancer.

Diabetic Nephropathy (DN) is a condition in which kidneys are severely affected over a while due to diabetes. This disease often progresses silently without showing any symptoms in the early stages but ultimately causes renal failure in the end stages. Zubiri *et al.,* 2014 performed proteomic analysis of urinary exosomes obtained from the patients of diabetic nephropathy and healthy donor controls [18]. They observed 254 proteins showing differential expression between these groups, and four proteins among them showed significant changes (aminopeptidase N, vasorin precursor, a-1-antitrypsin, and CP). These proteins were found to be involved in cell-to-cell communication and in regulating cell growth and cell death, thus opening a new area of research in the diagnosis of DN through urinary protein studies.

2.3 Bronchoalveolar Lavage Fluid (BALF)

BALF is a diagnostic technique in which sterile normal saline solution is introduced into the lungs to collect the airway lumen contents, with the help of a bronchoscope [19]. It contains the secretions, cells, and various proteins of the lower respiratory tract. Proteomic studies have identified around 1000 proteins in it and studied lung cancer and Chronic Obstructive Pulmonary Disease (COPD).

Lung cancer contributes to one of the highest mortality rates among various cancers. Lung adenocarcinoma is a type of lung cancer that originates from the lung tissue's glandular cells and is the most common form of it. Uribarri *et al.,* 2014 obtained BALF from patients diagnosed with lung cancer and control groups without disease [20]. Through proteomic studies, they identified 32 proteins that showed differential expression between the two groups. These proteins were involved in metabolism, cell death, inflammation, and cell proliferation, some of the classic mechanisms involved in cancer progression.

COPD is an obstructive disease of the lower respiratory tract, and cigarette smoking is the most common risk for developing it and may further lead to lung cancer [21]. Tu *et al.,* 2014 obtained BALF samples from COPD patients and healthy non-smokers [22]. Proteomic analysis identified 76 proteins in COPD smokers, which were different from that of healthy controls. These were involved in various biological functions such as inflammatory responses, glycolysis, alcohol metabolism processes, and oxidation-reduction. These studies concluded

that BALF proteomic studies could provide us with new biomarkers for these diseases using minimally invasive techniques.

2.4 Sputum

Sputum or phlegm is mucus from the respiratory tract (trachea and bronchi), and its composition changes if a disease or illness occurs [23] and is routinely used in various diagnostic tests. Titz *et al.,* 2015 used sputum samples from four groups – current smokers asymptomatic for COPD, smokers with early-stage COPD, former smokers, and non-smokers as control [24]. Using proteomic analysis, they identified several proteins which showed alterations in the current smokers vs. non-smokers groups. These alterations were in the mucin/trefoil proteins and prominent oxidative stress response. They also observed that former smokers showed almost complete attenuation for these changes, and their protein profiles were similar to those of non-smokers. Additionally, they noted some proteins had started showing some alterations in smokers' protein profile with early stages of COPD compared to smokers without COPD. Thus, it was possible to distinguish between various stages of smoking-related changes and understand the changes as the disease starts to progress using proteomic studies.

2.5 Cerebrospinal Fluid (CSF)

CSF is a clear fluid produced by the brain's choroid plexus, which fills up and surrounds the brain and spinal cord along with the extra-cellular Fluid of the central nervous system (CNS) [25]. Several studies have identified over 6000 proteins, including several protein biomarkers for neurogenerative diseases such as Parkinson's and Alzheimer's disease [26]. Khoonsari *et al.,* 2015 obtained CSF from patients with Alzheimer's Disease (AD) and non-demented patients as controls [27]. Proteomic analysis showed the downregulation of 8 proteins in the CSF of these AD patients compared to normal controls. These proteins played a role in biological functions such as synapse regulation, cell adhesion, migration functions, and immune response regulation.

Similarly, Sathe *et al.,* 2019 also performed proteomic analysis on CSF of AD patients and normal donors obtained through a lumbar puncture [28]. They identified 139 proteins which showed the change in the CSF of AD patients. Among these 139, 10 proteins showed significant differences, out of which three proteins were identified as potential biomarkers of AD. These were involved in neurodegenerative and cognitive functions.

2.6 Nipple Aspirate fluid

Nipple aspirate fluid (NAF) is a discharge fluid from the breasts through the nipple. It is secreted by the epithelial cells of the mammary ductal and lobular systems. It has various proteins, hormones, and carbohydrate components secreted exclusively from the breast and final form [29]. It can be collected quickly and noninvasively through modified breast pumps. NAF has been used to identify some protein biomarkers for breast cancer. Alexander *et al.,* 2004 used

NAF samples from women diagnosed with breast cancer and normal non-diseased controls [30]. They identified 41 proteins in control, compared them with breast cancer NAF samples, and identified two proteins with significant abundance in NAF breast cancer. These proteins were identified as acute phase reactant proteins and were found to have an immunomodulatory role.

2.7 Seminal Fluid

Seminal Fluid contains spermatozoa along with other components of the male reproductive tract. It is abundant with proteins that can serve as biomarkers in various diseases such as male infertility. Azoospermia, i.e., the absence of sperms in the seminal Fluid, is the most common reason behind male infertility. It can be Obstructive Azoospermia (OA) or Non-Obstructive Azoospermia (NOA), differentiated through surgical procedures for a conclusive diagnosis. Some researchers have performed proteomic analysis on seminal Fluid in the hope of distinguishing between various sperm conditions causing male infertility [31,32]. Batruch *et al.,* 2012 examined seminal plasma of NOA male infertility subjects and fertile males used as control [33]. Proteomic analysis showed the alteration of 52 proteins in NOA cases as compared to controls. Thirty-four proteins were upregulated in control groups and were involved in reproduction, the glycolytic pathway, and carbohydrate catabolic processes. Eighteen proteins, which were downregulated, play a role in glutathione metabolism and the glycolytic pathway. These studies aimed to differentiate the seminal fluid proteomics between fertile and infertile subjects and look out for new biomarkers that may help diagnose these conditions.

2.8 Amniotic Fluid

Amniotic Fluid (AF) fills up the amniotic sac and surrounds the growing fetus playing a protective and nutritive role. It contains various nutrients and antibodies, amino acids, hormones, lipids, etc. As the fetus grows, this Fluid also contains multiple proteins secreted by the fetus into it [34]. Proteomic studies on AF have identified some biomarkers of various fetal diseases and abnormalities, including some genetic diseases such as down syndrome. Down syndrome (DS) is a genetic disorder where trisomy of chromosome 21 occurs in the human genotype. Cho *et al.,* 2010 randomly collected AF from various pregnant women in varying age groups ranging from 30-45 years old [35]. These women later gave birth to normal children, but some fetuses were also diagnosed with down syndrome. The researchers divided the already collected AF into normal control and DS group. Through proteomic analysis, they identified 60 proteins that were altered in the DS AF samples. Some proteins were over-regulated and were found to be involved in protein degradation, hematopoiesis, development of reproductive system functions, and nutritional diseases. Some downregulated proteins were involved in ophthalmic and neurological processes. Out of these 60 proteins, they selected two proteins that were abundant in DS samples. One of them has its gene located on chromosome 21, and the second protein was involved in the development of multiple organs. They identified these two proteins as potential biomarkers of DS in amniotic Fluid.

2.9 Cervicovaginal Fluid (CVF)

The vagina and cervix are lined with a mucus layer originating from the cervix and various antimicrobial peptides that play a protective role. Vaginal Fluid is a complex biological fluid consisting of water, electrolytes, different organic compounds, a vast array of proteins, and proteolytic enzymes from plasma transudate, vaginal epithelial cells, and vaginal microbiota [36]. In addition to the contents of vaginal fluids, CVF may contain a part of the endocervix's secretions [37]. Proteomic analysis on CVF has identified some proteins that hold the capabilities to serve as biomarkers for some diseases such as anti-HIV-1 activity, cervical cancer, Intra amniotic infections [38].

Human Immunodeficiency Virus (HIV) has already killed 32 million people, and there is still no cure for it, and patients are only supported with life elongating therapies. ESNs (exposed seronegative individuals) are naturally resistant to HIV. Some health workers, children of HIV-infected mothers, commercial sex workers, intravenous drug users, or frequent blood transfusion receivers may be classified as the ESNs. Proteomic studies of the CVF lavage samples from ESNs have shown the presence of anti-HIV IgA and IgG antibodies, higher levels of suppressive chemokines, along with other anti-HIV proteins such as lysozymes lactoferrin, cathelicidin, and defensins [39,40].

Cervical cancer affects the cervix and starts with abnormal growth of cells, which can spread to other parts of the body, and it is the second most common type of cancer in women. HPV infection causes precancerous lesions and accounts for 90% of cervical cancer cases in women [41]. Van Raemdonck *et al.,* 2014, performed proteomic analysis on the CVF samples of women with HPV infection and precancerous lesions and identified six unique proteins which were exclusively found in those with these lesions and not in healthy samples [42]. Alpha-actinin-4 (ACTN4) was the most significant protein which was only found in the precancerous samples and has also been linked to other malignancies such as ovarian adenocarcinoma and oral squamous cell carcinoma. After several reproducible results, they concluded that ACTN4 could act as a biomarker indicative of persistent HPV infection causing precancerous lesions and cervical cancer.

3. PROTEIN STUDIES ON TISSUES OF VARIOUS ORGANS

Proteins pretty much portray the actual image of cellular events undergoing in the various parts of the body. Studying particular tissues can give us a closer look into different pathological processes [43].

The main advantages of performing protein profiling of specific tissues are; first, a high concentration of proteins can be obtained directly from the pathological site. Secondly, it gives us the ability to characterize the differences between diseased and normal tissue. Protein studies have been performed on the tissues of various organs such as the brain, heart, liver, prostate, ovaries, etc., with an

aim to have a better understanding of the mechanism of a disease or to find new biomarkers for its early detection or diagnosis.

3.1 Brain Tissue

The brain is one of the largest and most complex body organs and serves as the center of the nervous system in all vertebrates and most invertebrates. Some researchers have subjected brain tissues to proteomic studies in several neurodegenerative diseases such as Alzheimer's disease and Prion diseases [44].

Alzheimer's disease is a progressive neurodegenerative disease primarily affecting people in their older ages. It leads to several problems such as short-term memory loss, language problems, behavioral changes, and disorientation [45]. Abnormal protein traffic changes in the neurons also occur during the earlier stages of this disease. Donovan *et al.,* 2012, analyzed enriched membrane fraction of brain tissues from the deceased patients who suffered from AD [46]. They collected frontal cortex tissues from deceased AD patients during post mortem and normal non-diseased dead people to serve as control. Using proteomic studies, they identified a total of 1709 proteins from this tissue, out of which 13 proteins showed significant change in AD patients. This study was aimed to study the mechanism of disease progression and also to identify biomarkers in AD.

3.2 Breast Tissue

The breast is made up of a complex network of lobules, glandular structures, and branching ducts. Proteomic analyses have been performed on breast tissues to study diseases such as breast cancer [47].

Breast cancer is one of the most common types of cancers affecting women. It is often diagnosed during later stages of the disease when it is often too late to stop the malignancy from spreading. Though diagnosis is made by biopsies and other surgical methods, sometimes these methods are inconclusive when performed during the early stages of the disease. Wajeeh *et al.,* 2020, collected breast tissue samples from tumorous as well as adjacent normal tissues and subjected them to proteomic analysis [48]. They identified several proteins which showed differential expression during different stages of the tumor. Stage 2 tumor tissues had 12 proteins, and stage 3 tumor tissues had 17 proteins that showed changed expression levels compared to normal non-tumorous tissues. This study aimed to discover new biomarkers to identify the tumor stages of breast cancer and led to identifying various proteins that were unique only to a particular stage of the tumor and were not found in the other.

3.3 Heart Tissue

The heart is one of the vital organs of the body. Proteomic analysis has been performed on heart tissues by some researchers to study various cardiovascular diseases. Heart failure (HF) is one such disease with increasing incidences and

is characterized by poor prognosis and poor quality of life. It is caused due to the inability of the heart to pump with sufficient efficiency and thus leads to poor blood flow into the body. This often starts with structural and functional alterations to the left ventricle of the heart characterized as ischemic (ICM) and dilated cardiomyopathy (DCM), depending on the mechanism [49]. Roselló *et al.,* 2012 used proteomic studies to analyze patients' heart tissue suffering from HF (ICM and DCM) patients undergoing heart transplants and normal heart tissue from deceased people without any heart disease as controls [50]. They identified 11 proteins that were altered in heart failure patients. 10 out of 11 proteins were familiar to both ICM and DCM, and one protein - HSP70 (Heat shock protein 70) was found only in ICM patients. They also found that 7 out of the 11 proteins were involved in the cell death and apoptosis processes, hence establishing their role in heart failure progression.

3.4 Kidney Tissue

Kidneys are one of the organs which get affected due to diseases such as diabetes. Some researchers have performed proteomic analysis on kidney tissues to study diseases such as Diabetic Nephropathy (DN). This disease often progresses silently without showing any symptoms in the early stages but ultimately causes renal failure in the end stages. This makes its early diagnosis even more important to begin early treatment and change the patient's fate. Zubiri *et al.,* 2015 performed proteomic studies on kidney tissue of diabetic rats, and later on, human kidney tissues aimed to find any biomarkers even more reliable than albuminuria which is currently used [51]. They used diabetic rat kidney tissues along with normal non-diseased rat kidney tissues as control. Protein studies found that normal kidneys showed regucalcin protein presence, which was absent in the diabetic rat kidney tissues.

Regucalcin/Senescence Marker Protein-30 (SMP30) is an essential protein produced in the kidneys and liver which plays a pivotal role in calcium homeostasis in the body along with ascorbate biosynthesis and regulation of oxidative stress [52]. High sugar levels cause a decrease in the regucalcin levels in endothelial cells. Then, they performed protein analysis on the urine exosomes of these diabetic and control rats to see the presence or absence of this protein. It was seen that regucalcin was detected in the urine of the normal rat urine but was absent in diabetic rat urine. They further studied this in human kidney tissues and urine. Human kidneys were obtained from diabetic kidney transplantation patients, and age-matched normal kidney tissues were used as control. They found that regucalcin was reduced in kidney tissues of diabetic patients and was found in normal kidney tissues. Similar results were obtained in the human urine exosomes as well. So, using proteomic studies, they concluded that the role of regucalcin could be further explored as a potential DN biomarker along with albuminuria.

3.5 Liver Tissue

The liver is one of the essential organs found in vertebrates playing life dependant roles in detoxification of blood, protein synthesis, digestion, immunity,

and various metabolic functions. Some researchers have used protein studies on liver tissue in some diseases such as Non-alcoholic fatty acid liver disease (NAFLD). Yuan et al., 2020, performed proteomic analysis on liver tissues obtained from obese subjects, divided into metabolically healthy obesity (MHO) groups that show normal metabolism and diseased NAFLD groups when they were undergoing a weight loss surgery [53]. Protein studies identified various proteins showing differential expression in both MHO and NAFLD groups. One hundred thirty-two proteins (primarily involved in extracellular structure and matrix organization) were upregulated, whereas 184 (mostly involved in oxidative phosphorylation) were downregulated in the NAFLD group. This study aimed at a better understanding of the NAFLD metabolism changes.

3.6 Prostate Tissue

The prostate gland is the male reproductive tract's exocrine gland and secretes fluids that support the sperm by playing nourishing and protective roles. Prostate cancer is the most common non-skin cancer affecting males [54]. It is primarily asymptomatic in its earlier stages and progresses slowly and is often diagnosed later when it is often too late. Hwang *et al.,* 2007, performed protein studies on prostate biopsy sections to identify over 400 proteins, out of which PSA (prostate-specific antigen) was also determined [55]. They also identified the Proto-oncogene protein Wnt-3, which has a role in oncogenic transformation in prostate cancer [56]. Latonen *et al.,* 2018, performed proteomic analysis on the clinical tissue samples in different stages of prostate cancer and found differential expression of proteins in each stage [57]. Zhou *et al.,* 2019 performed proteomic analysis on prostate cancer tissues and non-cancer normal prostate tissues [58].

In comparison to normal prostate, low-grade prostate cancer showed differential expression of 54 proteins; high-grade prostate cancer showed 85 different proteins. There were six different proteins between low grade and high-grade types as well. There was upregulation of large putative protein-protein interaction (PPI) network in high-grade prostate cancer. This proteomic study served them to have a better understating of the regulation of proteins in prostate cancer cases.

3.7 Ovary Tissue

Ovaries are the primary female reproductive organs that produce and secrete the ovum. Proteomic analysis of ovarian tissues has been done better to understand some diseases, such as ovarian cancer. Ovarian cancer is the most lethal gynecological malignancy affecting women today [59]. The disease is often diagnosed in its later stages when it is too difficult to control. Ovarian cancer is a heterogeneous disease, i.e., it has various subtypes arising from different subtypes of ovarian tissues such as low grade and high-grade serous carcinoma (HGSC), mucinous, endometrioid carcinoma (EC), clear-cell carcinoma (CCC), squamous cell, transitional cell, mixed and undifferentiated tumors. Among these serous carcinoma is the most common and has lowest survival rate [60]. Some researchers have worked on ovary tissue proteomics to look for better

biomarkers and early detection. Wang *et al.,* 2012 performed a comparative proteomic study on ovarian cancer tissues and normal ovarian epithelial tissues [61]. They identified a total of 1259 proteins, 205 out of which were differentially expressed between tumorous and normal tissues. The majority of these were signaling proteins, transfer or carrier proteins, nucleic acid binding proteins, and oxidoreductase proteins. This provided them a look into the mechanism of ovarian carcinomas. Similarly, Wiegand *et al.,* 2014 used ovarian tissue samples of different tumorous subtypes – CCC, EC, and HGSC [62]. Proteomic analysis identified that CCC and EC had 50 proteins that were different from HGSC, and these were involved in regulating apoptosis, cell cycle, transcription, and other signaling pathways, giving a new basis for differentiating these subtypes and developing targeted interventions.

3.8 Uterus Tissue

The uterus is a secondary sex organ in the female reproductive system whose primary function is to support the growing fetus after fertilization of the egg with sperm. The uterus is lined by the endometrium layer. Endometrium tissue acquired through uterine aspirates has been used to study endometrial cancer by some researchers. Martinez *et al.,* 2017 used uterine aspirates from endometrial cancer patients and normal non-cancerous donors to do a comparative proteomic study and identified 28 proteins that showed higher activity in the endometrial tumor samples [63]. Nine proteins were different between endometrial EC and serous EC subtypes. Ceylan *et al.,* 2020 performed protein analysis on endometrial tissue obtained from the uterus of EC patients, and its healthy counterparts from donors were used as control [64]. They found that 11 proteins were different in the EC patients, and one protein was observed in the earlier stages but was not seen in the later stages of the disease. This study allowed them to better understand the progression of the disease.

3.9 Vaginal Tissue

Proteomic analysis of vaginal tissues has been used to study some pathologies such as PCV. Primary carcinoma of the vagina (PCV) is a rare disease affecting a limited number of women around the world. Due to its rarity, its etiology is not understood correctly [65]. Vaginal carcinoma also shows some similarities to cervical carcinomas; therefore, a correct diagnosis is essential to have a more targeted treatment. In an attempt to find novel biomarkers for vaginal carcinoma, Hellman *et al.,* 2004, used vaginal tissues from patients of PCV and cervical carcinoma and compared them with vaginal tissues from normal donors using proteomic studies [66]. They identified 67 proteins, 33 out of which showed differential expression in PCV and cervical cancer as compared to normal vaginal tissues. 23 out of 33 showed slight differences between PCV and cervical cancer, but overall, the protein profiles of both were similar. This was the first study to attempt proteomic studies on vaginal tissues to discover new biomarkers for vaginal diseases.

4. CONCLUSION

The utilization of proteomic studies has tremendous potential in a clinical setting. Human body fluids and organ tissues contain a wealth of protein components that can provide insight into the plethora of changes in their states both during normal and diseased conditions and can be used for early detection and effective diagnosis. It can help develop novel therapies for specific diseases by identifying particular proteins for that abnormality. It also offers a broader perspective regarding a disease by helping us understand its prognosis by studying the changes that occur during the disease compared to the normal state of the cell. Moreover, clinicians may also be able to tailor specific treatments for patients according to their proteome profile. However, there remain some challenges before these aspects of proteomics can be rationally incorporated into regular paradigms of diagnostic and therapeutic uses. There is a need to develop standardized models for the various processes crucial for proteomic studies, such as sample collection, preparation, and handling. Besides, the development of certified reference materials will bring uniformity for sample quality control and quality assurance and help eliminate possible anomalies in proteomic analysis. Researchers need to refine further the techniques used in proteomics to achieve reliable and reproducible results for patients of different ages, races, and other regions of the world. Nevertheless, despite these challenges, proteomic studies offer the promise of revolutionizing the healthcare practices and bring immense benefit in all aspects of patient care from diagnosis to prognosis and ultimately the successful treatment of diseases.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Chapter 12
Print ISBN: 978-93-5547-742-2, eBook ISBN: 978-93-5547-749-1

Comprehensive Review on a Complicated Endocrine Disorder (Acromegaly)

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DOI: 10.9734/bpi/cpms/v9/2457A

ABSTRACT

The purpose of this article is to make people familiar with signs and symptoms as well as treatment of acromegaly. Acromegaly is a disease or ailment that results in excessive bodily tissue growth and the malfunction of other metabolic processes. It is brought on by the hypersecretion of growth hormone from the anterior pituitary. Middle-aged persons are most commonly affected by acromegaly, which is typically brought on by a benign (non-cancerous) tumor. Patients with acromegaly have pain in the joints, physical deformities, deepening of voice, bulging chest, protruding lower jaw, large feet and hands, oily skin, vision disorder or erectile dysfunction (impotence). Acromegaly occurs after the fusion of growth plates while gigantism occurs before the fusion of growth plates. It is concluded that any disease management strategy must focus on the needs of patients and do everything possible to improve patients' healthcare experience and minimize their treatment burden. The present study may be helpful for a systematic and targeted research for more convenient management of acromegaly.

Keywords: Acromegaly; growth hormone; pituitary adenoma; Insulin-like Growth Factor-I (IGF-I).

1. INTRODUCTION

Acromegaly is a unique illness brought on by excessive growth hormone synthesis, typically from an adenoma of the anterior pituitary gland [1]. The people that are diagnosed by this disease are mostly of 40 years, with equal number of men and women. The disease is exceptional and the prevalence is not clear; a recent review noted estimates of between 40 and 130 cases per million adults [2]. An authenticate diagnosis of acromegaly generally takes several years, that may lead to serious consequences for patients' health. The delay in the diagnosis may be due to the similar symptoms of acromegaly with that of hypertension and diabetes [3]. Endocrine disorders can influence the

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haemostatic balance. Abnormal coagulation test results have been observed in patients with abnormal hormone levels. Also unprovoked bleeding or thrombotic events have been associated with endocrine disease [4].

The purpose of this article is to make people familiar with signs and symptoms as well as treatment of acromegaly. Acromegaly is associated with multiple comorbidities, such as diabetes mellitus, sleep apnea, arthropathy, cardiovascular system disorder (e.g., hypertension) and menstrual irregularities. Early identification of acromegaly facilitates prompt treatment initiation and may minimize the permanent effects of excess growth hormone. The primary treatment for many patients will be pituitary surgery, although not all patients will be eligible for surgery or achieve a surgical cure. If biochemical control is not achieved following surgery, other treatment options include medical therapy and radiation therapy [5,6]. It is expected that the familiarity with the signs and symptoms of acromegaly will facilitate early evaluation and management of the disease [7,8]. Acromegaly cannot be prevented but early treatment may prevent the disease from getting worse and help avoid complications.

2. ETIOLOGY

There are so many causes of acromegaly which can be divided into three parts: primary Growth Hormone (GH) excess, ectopic GH excess as well as excess Growth Hormone-Releasing Hormone (GHRH). Most acromegaly cases are caused by non-cancerous (benign) tumour (adenoma) of the pituitary gland. The adenoma produces too much growth hormone. The most commonly associated mutation involves activation of the alpha subunit of the guanine nucleotide stimulatory protein gene [9].

There are other causes of acromegaly that involves over production of GHRH. These can be divided into two parts i.e, peripheral cause and central cause. Peripheral cause involves the secretion of GHRH from small cell lung cancer, bronchial carcinoid tumours, adrenal adenoma etc, while central cause consists of hypothalamic hamartomas, choristoma and ganglioneuroma [10].

3. EPIDEMIOLOGY

Acromegaly is an uncommon disease with an ubiquity of 4,600 per million population and approximately 116.9 new cases per million per year. The average diagnostic age of acromegaly is about 40 for men while 45 for women [11,12].

Acromegaly generally appears in the 3rd decade of life. According to a current research in Belgium, pituitary tumour may be more frequent and the generality of acromegaly would be approx.

100-130 cases per million inhabitants [13]. A lot of recent epidemiological studies have been carried out in Germany [14] on the detection method for acromegaly. Application of systemic measurement of Insulin-like Growth Factor-I (IGF-I) in primary care patients in the general population one day discovers the epidemic of

biochemistry. It has been observed that acromegaly is even greater (1,043 parts per million).

4. PATHOPHYSIOLOGY

The largest gland of body (pituitary gland) is located at the bottom of the brain and is responsible for secreting many hormones, including GH, which is regulated through complex feedback mechanisms. Acromegaly is usually due to the presence of (benign) pituitary tumours. Depending on its size and location, the tumour mass may cause problems, such as loss of vision due to compression of the optic chiasm. However, tumours also secrete excessive amount of Insulin-like Growth Factor-I (IGF-I). Long term excess of GH and IGF-I can cause a variety of important comorbidities, including cardiovascular complications, cerebrovascular events, gonadal dysfunction, glucose intolerance, diabetes, sleep apnea, impaired respiratory function, colon tumours and bone and joint disease [7,15-17].

GH overproduction is also associated with increased mortality. If there are complication, especially cardiovascular disease, mortality will increase further [18]. Specially, the death rate of untreated acromegaly is two or three times than that of the general population. Hence, early evaluation and biochemical normalisation are essential to minimize the permanent life-limiting effects of excessive GH.

5. SYMPTOMS

Acromegaly can cause a number of symptoms, such as bad odour and sweating mainly at night); headache, altered size of pituitary adenoma, acral paraesthesia and pain in joints. It was also observed that the sound gradually deepened (Fig. 1).

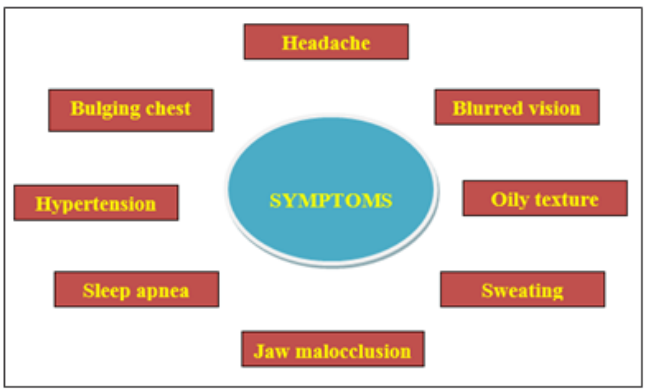

Fig. 1. Different symptoms of acromegaly

6. DIAGNOSIS

Patients with long-term illnesses usually appear in the later stages with significant physiological features (e.g, enlarged hands, feet, lips and tongue; supraorbital bulge and protrusion of lower jaw) [19].

However, the appearance of the physical changes is insidious, yes, and patients are less likely to have complaints directly related to these characteristics of acromegaly; instead, they are more likely to show other diseases that are more common in primary care (e.g, cardiovascular disease, diabetes, hypertrophy, blood pressure and sleep apnea) (Fig. 2).

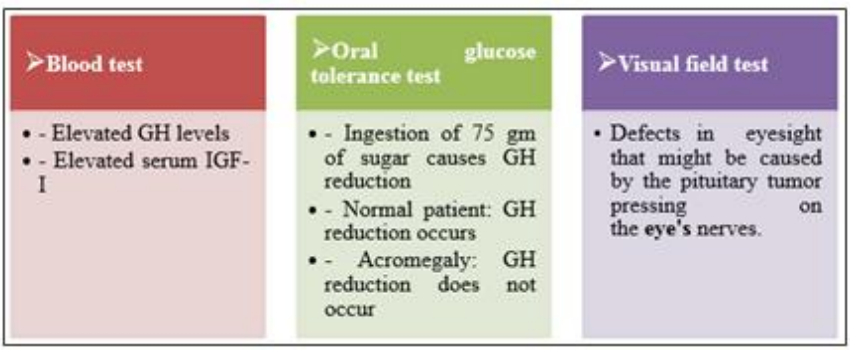

Fig. 2. Diagnosis procedures for acromegaly

6.1 Treatment and Management

The purpose of treatment of acromegaly includes the control of biochemical parameters (GH and IGF-I levels) and related signs and symptoms, the influence of local tumour masses, the treatment of comorbidities and the improvement of death rate [20].

Surgical therapy: Surgery is optimal treatment for all large microversometers and adenomas, which can cause massive effects. Adenomas can also be performed as a method that can be performed and is likely to occur somatic cure. The best predictors for surgical healing include smaller tumours sizes, lower level of GH/IGF-I and surrounding structures (such as cavernous cavity). The study also showed that treatment with neoplastic medications with octreotide before surgery could increase the remission rate (Fig. 3). However, more research is required to determine if this should be done daily or can benefit from this approach. In general, this type of surgery must be carried out in a central part with an experienced pituitary neurosurgeon which performs at least 50 operations [21].

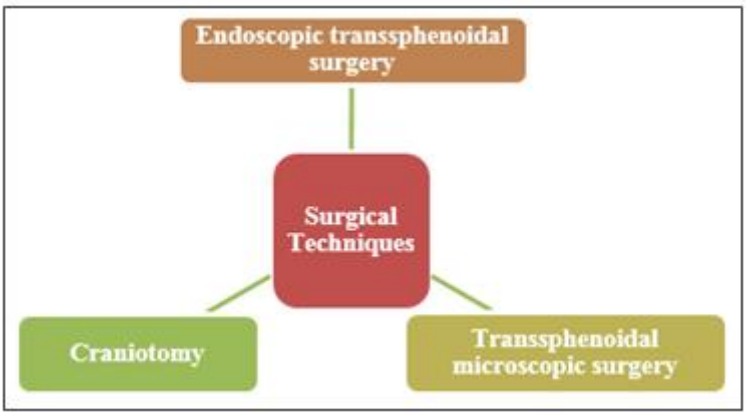

Fig. 3. Available surgical treatment of acromegaly

Medical therapy: The drug is suitable for patients who do not want surgery. The risk of surgery is too high and it is not suitable for surgery, because the tumour may not be removed after the initial surgery and the patient may reappear, but may not meet the conditions for repeat treatment. As mentioned above, preoperative drugs can also work. These are as follows [22-24].

1. Somatostatin analogues
   I. Octreotide
   II. Lanreotide
   III. Pasireotide
2. Dopamine receptor agonists
   I. Cabergoline
   II. Bromocriptine
3. GH – Receptor antagonist
   I. Pegvisomant

Management of acromegaly in pregnancy: GH secretion during normal pregnancy will vary. GH-secreting tumours have oestrogen receptors, especially those that secrete prolactin. The concern is whether the pregnancy status will increase the size of the tumour; however, in some studies, it has been found that most women do not experience significant changes in tumour size during pregnancy. However, since the risk still exists, women should be closely monitored through continuous visual monitoring [20,24]. Radiotherapy: Treatment with radiation is preferred when patients are not able to cure with medication/drugs or via surgical method. The patients that are getting radiotherapy are monitored for hypopituitarism [25].

Stereotactic radiosurgery: This is a precise radiotherapy that targets high doses of radiation at the tumour and minimises the risk to nearby healthy brain

tissue. It is a single high-dose radiation. The adenoma must be a few millimetres from the optic nerve abyss to avoid damage caused by this technique. in the absence of drug treatment, the remission rate of this method is 17% to 50%, and the time ranges from 2-5 years. This technology has more advantages than traditional fractional radiation therapy, which can provide better targeting and reduce radiation exposure to surrounding tissues, and shorten the time to control IGF-I and GH levels [25-27].

7. CONCLUSION

Acromegaly is a serious disease associated with multiple comorbidities with and increased mortality. The delay in diagnosis is usually very long. This may be due to the lack of knowledge of the disease by health professionals, insidious episodes of different characteristics and possible typical complaints of patients with other more frequent disease in primary care. Health professionals who understand acromegaly can help alleviate this delay by identifying the signs and symptoms of the disease early. Treatment can begin immediately, which can reduce mortality.

For patients, the burden of disease and treatment is enormous. Often, more than one type of treatment is required, and long-term medication is often required. Comorbidities, especially those of a cardiovascular nature, may also require immediate attention. Even if the underlying hormonal abnormalities are successfully managed, several comorbidities will still exist, requiring additional long-term follow-up and treatment. It is clear that the care of these patients requires a highly coordinated multidisciplinary approach. It is also clear that any disease management strategy must focus on the needs of patients and do everything possible to improve patients' healthcare experience and minimize their treatment burden. The present study may be helpful for a systematic and targeted research for more convenient management of acromegaly.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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DISCLAIMER
This chapter is an extended version of the article published by the same author(s) in the following journal.
Journal of Physical Medicine Rehabilitation Studies & Reports, 3(3): 1-3, 2021.

Chapter 13
Print ISBN: 978-93-5547-742-2, eBook ISBN: 978-93-5547-749-1

Has the DSM Failed?

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DOI: 10.9734/bpi/cpms/v9/6743F

ABSTRACT

The DSM's atheoretical and consensus-driven diagnostic classification system has become increasingly problematic to implement into clinical practice, by creating diagnostic confusion between diagnoses. . Neither has it properly incorporated decades of research on etiology, in order to inform diagnostic clarity. Instead it instructs clinicians to use algorithmic/iterative methods of making diagnoses, contrary to longstanding clinical training traditions.., Academic institutions in the United States are now mostly reliant on the DSM. making it a paradoxical impediment to the authentic understanding of psychiatric disorders as well as their treatments.

Keywords: DSM; psychiatric disorders; schizophrenia; etiological factors.

1. INTRODUCTION

It was originally hailed as the psychiatric profession's systematic break from the clutches of psychoanalytic thinking in order to lend proper credence to psychiatric diagnoses. Based upon seminal work [1,2], the DSM III-R was launched as the manual that would improve inter-rater reliability (as well as validity) of all psychiatric diagnoses, whether they were made in New York, London or anywhere else in the world [3]. Later iterations followed, including DSM IV, DSM IV-TR and most recently the DSM-5 in 2013 [4].

What has been gleaned in the decades that have followed? This is an obviously broad question, and depends much upon which country and which systems the DSM is pertinent to.

For example, in the United States, a whole billing and coding infrastructure has grown up around this iconic manual [5], that relies upon it (though not exclusively, as the ICD has remained the bedrock of diagnostic coding in the United States [6]). Hence, insurance companies, physician's offices and hospitals would not likely earn revenue, were it not for the existence of the DSM.Teaching

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curricula of residents and medical students, as well as most psychiatric textbooks have included the DSM as the backbone of their content. Then there is the pharmaceutical industry, which has geared much of its efforts toward FDA approval of psychotropic drugs, based upon 'established' diagnoses such as bipolar depression even if some these indications were statistically questionable [7]. Not surprisingly, academic institutions in the United States have relied almost exclusively upon the DSM to focus their efforts on furthering knowledge about various psychiatric disorders as well as treatments.

Lastly (and despite its disclaimers) the DSM right has been brought right into the courtroom, in order to help address important legal questions such as insanity, civil commitment and competence to stand trial [8]. Thus, the importance of the DSM (at least in the United States) cannot be overstated. Then why would one ask if it is has 'failed'? This is a controversial question, given its near ubiquitous use.

The answer may lie more in the day to day clinical realities of clinical practice, than with the institutions referred to above. For it is here that diagnoses are regularly made. As has been learned over decades, the various disorders in the DSM are not as clearly distinguishable as they may appear [9]. Given that the criteria have been revised over this same time frame by repeated consensus and collaboration, this may not be a surprising finding.

However, of growing concern has been the use of the "Not Otherwise Specified" category that was noted to occur with almost alarming frequency in the years following the publication of the DSM-IV [10]. What does this signify? It is perhaps a genuine reflection of the limitations of the categorical classification of psychopathology with respect to real life clinical practice.

Studies have also revealed that the DSM's categorical classification of psychopathology, with its arbitrary rules for establishing diagnoses from various criteria, is not how clinicians actually arrive at diagnoses [11]. Not surprisingly, and in complete contrast to how DSM diagnoses are arrived at, most medical school teaching still teaches deductive reasoning and pattern recognition of a constellation of symptoms, more commonly termed a 'syndrome' [12,13].

Medical school also tends to emphasize etiological factors at the root of such symptoms, thereby making them more intelligible to those wanting to learn more about such psychopathology. An example would be anxiety-provoking cues (of emotional salience) triggering behavioral inhibition and leading to avoidance behaviors in anxiety disorders (with corresponding neurobiological pathways that would correlate with such reactions and behaviors).

Yet, the DSM has never made adequate use of such cumulative knowledge, somehow 'expecting' clinicians to adapt to a more algorithmic/iterative way of arriving at a diagnosis. Hence the much famed "256" ways to arrive at a diagnosis of Borderline Personality Disorder [14]. What was therefore originally

carved out of diagnosis as an aberration (etiology), arguably 'hollowed out' the clinical 'essence' of these disorders for practicing clinicians [15].

The overlapping, "co morbid" disorders are similarly important in this context. This is because the anxiety disorders are not only etiologically, but also phenomenologically linked. Thus, the ruminations and avoidance behaviors of social anxiety disorder are also commonly found in combination with many symptoms of a "co-morbid" generalized anxiety disorder [16].

Similar phenomenology has been found in Post-Traumatic Stress Disorder and Obsessive Compulsive Disorder. However, they have now been curiously 'decoupled' from the anxiety disorders in DSM-5 [4,17]. This again speaks to the rather arbitrary and unwarranted delineation of these disorders, based upon 'splitting' of shared phenomenology.

Then there is the problem of 'lumping' of certain subtypes of disorders. With respect to Schizophrenia, subtypes in DSM IV were eliminated in DSM-5 as lacking reliability as well as prognostic significance [18]. Yet, for many practicing clinicians, paranoid schizophrenia remains very obviously distinguishable from other subtypes of Schizophrenia, with several (albeit older) studies indicating its better clinical prognosis [19,20].

Similarly, with respect to Autistic Disorder and Asperger's Disorder, the obvious clinical distinctions between these two have been 'lumped' together into the all encompassing 'Autism Spectrum Disorder' [15]. Unfortunately, service eligibility considerations and alarm at the rising incidence of autism disorder rather than strict clinical considerations appeared to be behind this particular change [21].

With respect to the clinically critical delineation of ADHD from Anxiety Disorders (a conundrum which outpatient psychiatrists are presented with regularly) there again appears to be inadequate diagnostic guidance by the DSM. Thus, being 'keyed up' and being 'on the go' can look phenomenologically identical, as can forgetfulness due to inattention.

Perhaps of greater utility (for clinicians) would be the more intense subjective distress and discomfort experienced with anxiety than with prototypical ADHD, or the predisposition to subjective misinterpretation of everyday events because of the degree of hypervigilance and relative distractibility attributable to hyperarousal with anxiety.

This is because the 'neo-Kraeplinian' model (from which DSM was spawned) emphasizes empirical concepts such as course, and prognosis, rather than symptom complexes per se [22]. This is in contrast to the Jasperian model which, coming from an existentialist tradition, was more geared towards elucidating a patient's subjective experience of psychopathology [23].

Guidance by the DSM in distinguishing disorders has therefore been distinctly lacking, particularly in the area of psychopathology. Thus, the distinct "whatness"

of a disorder is now absent from its current nomenclature [24]. In so doing, the DSM could have also helped clinicians fashion differing pharmacological approaches, by relating now-established knowledge about known neurobiological pathways [25].

For patients, the unintended consequence has been a plethora of labels added as codes to a "bill". This may paradoxically worsen stigmatization of psychiatric disorders. This is not to detract from the subjective distress and dysfunction experienced by the patient (something the DSM was right to emphasize). However, as a result of such dry 'lists' of symptoms/disorders being ubiquitously available on the internet, and without any corresponding discussion of etiology, patients are oftentimes left more overwhelmed with disjointed facts than actually educated.

Perhaps the greatest confusion lies in the various subtypes of Bipolar Disorder. Here, the mixed bipolar category appears to resemble more of an agitated depression than a separate subtype at all. It is now clear that Bipolar Disorder is one of the least well described disorders [26], especially in the pediatric age group [27]. Yet its overdiagnosis and misdiagnosis has been well described [28,29].

From extensive post-DSM IV publication research, the Schizoaffective Disorder diagnosis similarly appears to be on rather shaky phenomenological ground [30]. Yet, it too has been diagnosed with alarming frequency, perhaps because it inadvertently 'lumps' psychotic and mood symptoms so successfully together.

With respect to the personality disorders, DSM-5 appears to have stymied a timely opportunity to facilitate a radical paradigm shift from the trait and criteria-based model to a more dimensional model of personality functioning.

Despite over two decades worth of data validating the utility of personality dimensions, including work on various temperamental as well as cognitive aspects of personality [31-33] we now have to wait yet longer for the next iteration of DSM to see if such dimensional descriptions can be shifted over to the mainstream personality disorder section. Meanwhile existing (and flawed) categories become even more entrenched [34].

Then there is the relational context. By aligning itself so narrowly to an exclusively medical model of mental disorders, DSM successfully marginalized psychological or relational aspects of psychopathology, confining these to various appendix sections ("Other conditions that may be a focus of clinical attention") [35]. A good example of this would be Intermittent Explosive Disorder, where neither the DSM IV nor DSM-5 considered the relational context, implying that this was merely derangement of impulse control ('behavioral outbursts'), and arbitrarily assigning the frequency to twice a month [36].

DSM IV did at least devote an entire Axis (IV) to such 'psychosocial stressors', but this has now been eliminated in DSM-5, as has the degree of

decompensation (Axis V) that used to be reflected in the GAF score. This would appear unhelpful for both clinicians and patients, because it eliminates focus on the impact of psychiatric morbidity as well as ongoing stressors as they pertain to daily psychosocial functioning.

Defensive functioning was also retained in recognition of a psychodynamic, yet clinically applicable paradigm for the DSM. Yet, in reality, its use was rare by clinicians and not even recognized by insurance companies. As a result of insurance-driven incentives, clinicians were only encouraged to record Axis I entities that were more likely to be reimbursed (something that defensive functioning, relational problems and other clinically relevant codes such as 'Non Compliance with Treatment' were not) [5,26]. This would similarly explain the underutilization of the Borderline Personality Disorder diagnosis, in favor of the 'billable' Axis I Bipolar Disorder, over the course of the last decade [21].

The remaining serious criticism (including from a former DSM taskforce chairman) has been an unwarranted expansion of mental disorders that has arguably pathologized various behaviors within the spectrum of normal mental health [37-39].

So, did the DSM succeed at all? In 'dethroning' the prevailing unscientific and speculative ideology of psychoanalytic theory, and in conjunction with subsequent biological advances in the field, the DSM did appear to transform psychiatry into a more credible 'medical' specialty. And though it promoted an almost exclusively biological model of psychiatric disorders, this was nonetheless helpful in enriching understanding of disorders such as Schizophrenia and Bipolar Disorder, for which their biological basis is now indisputable.

More recently, DSM-5 was astute to add a developmental perspective, proceeding pragmatically through disorders relevant to appropriate stages of the human lifespan. Certain disorders have also received appropriate revisions. For example, Post-Traumatic Stress Disorder has criteria have now been more parsed out to include explosive anger as well as dissociation (including the complex PTSD subtype) [40].

The DSM is now firmly embedded in the healthcare landscape, and looks likely to stay, albeit with iterative changes in subsequent editions. In its lifespan, it appears to have gone on to serve four major stakeholders: academia, big pharma, insurance corporations and finally clinicians. Of these, it is the last category that is arguably the least well served. Sadly, it is also the group that advocates the least well for itself by virtue of its being so diverse, so mired in day to day patient care, and by its not being as politically 'galvanized' as the other stakeholders above [41].

By its very association with corporate, government and academic entities, DSM has gone on to achieve almost 'monolithic' power [42]. Yet, paradoxically, from clinicians' vantage point, it may have also become its own barrier to effective mental health care. This is because the prevailing emphasis on 'co-morbidities'

inevitably leads to unintended, 'downstream' consequences, such as polypharmacy as well diagnostic confusion for both clinicians and patients alike.

The notion of reliable diagnoses and good inter-clinician communication was always a laudable one. But, as research has shown, this almost exclusive focus by the DSM has actually led to poor emphasis on the validity of psychiatric diagnoses [43,44] as well as the progressive erosion of clinical psychopathology [45].

Hence, a long overdue appeal 'from the clinical trenches' is being made: Let us make our diagnoses, our descriptions and our distinctions mean something once more. Let us also put our patient's concerns in proper context, thereby utilizing the biopsychosocial approach that we have given so many platitudes to heretofore. The plea is thus: Psychiatry (and by extension, its professional 'arm' known as the DSM) should not unwittingly end up complicating the very disorders that it is trying so hard to treat.

2. CONCLUSION

The DSM serves as the foundation of most psychiatric textbooks and teaching programmes for residents and medical students. Since the DSM has always 'expected' physicians to adapt to a more algorithmic/iterative approach of making a diagnosis, it has never made full use of such cumulative information.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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Research and Academic Experience: May 2017 Member, Editorial Board, World Journal of Psychiatry and Mental Health Research.
2011-2021 Member, International Advisory Board, Australian & New Zealand Journal d Psychiatry.
Added Qualifications in the Subspecialty of Forensic Psychiatry.
American Board of Psychiatry and Neurology, Inc Certificate Number: 1696.
Ten Year Re-certification Examination April 2019.
Certified: 2009.
Diplomate in Psychiatry American Board of Psychiatry and Neurology, Inc.
Certificate Number: 54626.
Ten Year Re-certification Examination February 2015.
Diplomate in Psychiatry.
American Board of Psychiatry and Neurology, Inc.
Certificate Number: 54626.
Certified: 2005.
Part I Examination - November 2004 (first attempt).
Part II Examination - April 2005 (first attempt).
Oct 2017: "Violence Against Psychiatrists: Psychodynamic Perspectives" Presentation at the American Academy of Psychiatry and the Law Annual Meeting.
Mar 2001: "Towards an Integrated Phenomenology and Biology of Deviant Sexuality" Southern Association of Research Psychiatry Conference Presentation.
Aug 2008: "Ten Tips for Parenting your Teen" Xlibris Publishing Corp. Philadelphia, PA.
May 2017- June 2022 (also July 2013 - December 2016).
Forensic Psychiatrist, Northern Virginia Mental Health Institiute Clinical Assistant Professor, George Washington University (GWU) School of Medicine.
October 2009 – September 2010 Medical Director, Northern Virginia Mental Health Institute (NVMHI), Falls Church, Virginia;
Clinical Assistant Professor, George Washington Department of Psychiatry and Behavioral Sciences.
September 2008 - October 2009 Medical Director/Supervisory Medical Officer,
Civil Psychiatry Programs, Saint Elizabeths Hospital, Washington, DC Faculty Member, Saint Elizabeths Residency Program in Psychiatry.

Formal Psychiatric Training:
July 2003 - September 2003
(Jan 2002 - June 2002 - counted toward completion of adult psychiatry residency training).
Fellowship in Child Psychiatry
Division of Child Psychiatry, University of Virginia Health System

July 2002 - June 2003
Fellowship in Forensic Psychiatry
Institute of Law, Psychiatry and Public Policy
University of Virginia Department of Psychiatric Medicine and School of Law

June 1998 - June 2002
Residency in Psychiatry (incl. 6-month child psychiatry fellowship –please see above)
Department of Psychiatric Medicine, University of Virginia Health System

August 1993 – January 1995 Psychiatry Training Program
Department of Psychiatry, University of Cambridge (UK)

MBBS (equivalent of the MD degree in the United Kingdom) University of London (July 1992)
United Medical and Dental Schools of Guy's and St. Thomas' Hospitals, London (UK).
(Now known as Kings' College London, GKT School of Medical Education)

B.Sc. (B.S.) - Upper Second Class Honors University of London (June 1990)
Glaxo-Wellcome Institute for the History of Medicine University College London (UCL), University of London (UK)

May 2006 President, Tristate India Association of KY,OH and WV
Jan 2005 Secretary, Tristate India Association of KY,OH and WV
May 2000 Psychiatry Resident Representative, University of Virginia Health System
April 2000 Recitation, "Cheltenham's Ladies" Virginia Festival of the Book
Sept 1989 President, International Hall of Residence, University of London
Sept 1988 Vice-President, International Hall of Residence, University of London

Research Area: His Research Area includes Diagnostic clarity; psychopathology of anxiety, anger and aggression; distinguishing ADHD and anxiety.

Number of Published Papers: He has 15 Published papers

Special Award: 2015-2016 Certificate of Appreciation for Dedication and Excellence in Teaching, George Washington University Dept of Physician Assistant Studies.
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ACKNOWLEDGMENT: This chapter is a reproduction of an editorial , originally published by the same author(in the World Journal of Psychiatry and Mental Health Research, 1(1): 1003, 2017.

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