Editorial

BEST PRACTICE FOR GLOBAL IMPACT; FOSTERING COMMUNITIES OF PRACTICE THROUGH MEANINGFUL NETWORKING

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Communities of practice (CoP) are mainstay in identity formation of any profession[1]. Networking events and conferences are by far the best way to gather together people of similar interests from diverse backgrounds to foster a kinship that not only leads to advancement of the profession but also of the participating individuals. Organizations and societies hosting international conferences and meetings through quality interactions generally succeed in building long term connections projecting a soft image and creating goodwill. Health professions education (HPE) also known as medical education has gained momentum in Pakistan in the last 20 years with start of masters, and doctorate programs in major universities around the country. The growth of the specialty has resulted in an increase in local scholarship and also in rethinking the curriculum and assessment of the health professions, especially the field of medicine. International conferences like Association of Medical Education of Europe (AMEE)[2] have generated momentum of medical education fraternity coming together from around the globe and this has resulted in conferences and seminars being conducted by colleges and universities at local level as well to create a dialogue and develop a culture of quality educational reform in Pakistan. One of the conferences that has stood out in the last decade is the International conference on Health Professions Education (ICHPE)[3] which started as a small HEC funded event in 2014 attended by the delegates from one private university and expanded in 2023 to one of the largest medical education conferences held at national level with collaboration of 25 institutions including the public and private sector, boasting the biggest sectoral collaboration in the field of health professions education in Pakistan. Keeping up with tradition, the famous national and international speakers at ICHPE in year 2023 stressed the adaptation of medical education to the local context and incorporating cultural, social, and ethical dimensions in HPE was emphasized throughout the sessions. The theme resonated with the current pulse of medical education, highlighting the need for a holistic approach to healthcare training. The conference brought together renowned 19 national and 17 international speakers who shared their insights and experiences, enriching the sessions through sharing of best practices in HPE around the world. The presentation of quality educational research by the medical education fraternity, the workshops and micro-credentialing courses like leadership and governance practices brought the novices and the experts in the field closer together thus providing platform for movement of peripheral observers to central participants within the

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health professions education community. This remarkable growth of ICHPE can be attributed to focus of the event on connecting international experts in medical education to the national stakeholders including not only the students of health professions education but also the heads of institutions, accrediting bodies and the governmental organizations aiming to appraise the guests with the best practices in the country and building a rapport to enhance the image of our graduates in the eyes of the important world bodies. This thoughtful interaction over the years has resulted not only in communicating the Pakistani context to the international education arena but has also contributed towards the world accepting the implementation of standards of medical education that could be contextualized to needs of local community. The national leaders in medical education and stakeholders in government and accrediting bodies like Pakistan Medical and Dental Council (PM&DC) have also gotten a chance to be able to connect to the presidents of international organizations like world federation of medical education (WFME) specifically in 2022 and 2023 and have subsequently ensured that the country is well prepared for taking appropriate measures to maintain eligibility of our medical graduates to be part of the healthcare workforce around the world. Communities of practice are groups of individuals who have common concerns, problems, and passions with an intent to enhance their expertise and knowledge by interacting on a regular basis.[4] This can be successfully achieved through openness and shared ownership of any networking event. Conferences ensure diversity that then translates into quality outcomes. Focus on developing guidelines and policies that are co-created by local and international experts and are openly shared with all from faculty to students to organizational leads has built trust in ICHPE as a platform for knowledge gaining and sharing. The growth of any community and its regular interaction leads to sustainable growth in higher education. The conferences that ensure advancing country wide agendas, encourage research, ensure community outreach, and foster a culture of knowledge dissemination are instrumental in becoming the important tools to ensure achievement of sustainable development goals[5]. ICHPE in its upcoming years aims to maintain the standards and expand its horizons from professional to interprofessional education that can bring together experts not only within health professions but also across the cutting-edge worlds of technology and artificial intelligence to be able to navigate the latest industrial revolution for better quality of healthcare education leading to a personalized delivery of quality healthcare to the masses. In the words of Brian Tracy, Continuous learning is the minimum requirement of success in any field.

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Original Article

DIAGNOSTIC ACCURACY OF MICROSCOPY VERSUS PCR TECHNIQUE FOR THE DETECTION OF PLASMODIUM SPECIES IN PAKISTAN

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Abstract

Background: According to latest data available Plasmodium vivax and Plasmodium falciparum are the most common species of plasmodium present in Pakistan. This study focuses on the current status of malaria specie distribution across different provinces of Pakistan.

Material & Methods: This is a cross sectional study which is community based it was carried out in endemic areas of 04 provinces of Pakistan. The study was conducted stepwise by first microscopically confirming Plasmodium-positive blood samples and later these samples were reconfirmed by polymerase chain reaction (PCR) specie specific for detecting four species of human malaria.

Results: Total of 450 PCR-positive samples were collected amongst these 29 (6.4%) were P. falciparum, 386 (85.8%) were P. vivax, and 35 (7.8%) were mixed P. falciparum and P. vivax. Total 39 (8.7%) P. falciparum, 393 (87.3%) were P. vivax and mixed infections were (18%) positive in microscopically. There were no positive cases of Plasmodium malariae and Plasmodium ovale.

Conclusion: According to the study findings P. vivax and P. falciparum are most prevalent plasmodium species in in Pakistan, in addition mixed infections were also contributing to malaria prevalence in Pakistan. Regional variation in the prevalence and species composition was also found in the study.

Key Words: Plasmodium falciparum, Plasmodium vivax, Malaria, Pakistan

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INTRODUCTION

The bite of infected Anopheles mosquito causes Malaria in humans which is a life-threatening disease. Malaria is preventable and curable disease. According to reports of 2017 there were 219 million cases of malaria in 90 countries due to which death toll reached 435,000.[1] WHO reports label Pakistan as a country with highest load of malaria in EMRO region, the number of cases reported are one million annually. Some developing countries Sudan, Yemen, Somalia and Afghanistan show the comparable number of cases as of Pakistan.[2]

Different approaches are presently tried to improve malaria control activities which ultimately are intended towards the eradication of this disease. Total of four types of Plasmodium species cause malaria in humans which are P. falciparum, P. vivax, P. malariae and P. ovale. Another specie P. knowlesi, has been discovered in Southeast Asia which causes malaria in humans.[3] Plasmodium knowlesi is more lethal due to its rapid clinical course so early diagnosis and treatment can prevent

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mortality.[4,5] In Malaysia microscopy is the method of choice for diagnosis but the limitation for P. knowlesi is that its ring stage resembles P.falciparum and in later stages the trophozooite resembles with P.malariae,[6,7] so the chances of wrong diagnosis increase. Early diagnosis and treatment in cases of malaria is the most effective way in to reduce malaria cases and burden of disease.[8]

Malaria is considered to be very intense public health issue which is common in subtropical areas of the world. Malaria is caused by mosquito having five different types. The first-line treatment depends on the type of parasite causing the disease, proper selection of anti-malarial drug is needed according to the specie seen on microscopy. Correct identification on microscopy is needed before starting the antimalarial. The common and less expensive method which can be easily followed in endemic areas anywhere is through Giemsa-stained thick blood smears by microscopy.[9]

Molecular diagnostic methods are more sensitive tests for the diagnosis of species as compared microscopic tests, however the technical expertise of the investigator is more important for the specificity & sensitivity of the test. On microscopy only half the number of patients is correctly diagnosed as compared to the molecular diagnostic methods such as polymerase chain reaction (PCR) in endemic areas.[10] Some species of Plasmodium like P. knowlesi and P. malariae, are difficult to differentiate from each other on the basis of morphological features. Cases of P. knowlesi in Malaysia and other East Asian countries are recently reported in spite of the fact that first confirm case of human P. knowlesi infection was reported in 1965. Retrospective studies done in Malaysia during 2004 made it clear that microscopy alone has given wrong diagnosis about P. knowlesi human infection cases as P. malariae.[11]

To detect Plasmodium species in human nested PCR or semi-nested PCR tests are done but none can correctly detect the separate the five species of plasmodium. As the simple molecular diagnostic method is more sensitive as compared to microscopy therefore it needs to be introduced immediately to differentiate between the different species of Plasmodium parasite.[12] 374,513 diagnosed & confirmed cases of malaria were reported among the public and private hospitals of country and so the total disease burden across Pakistan is calculated. Screening was done for 6.5 million malaria suspects at these health facilities. The number of diagnosed cases for (PV) 84.0% that is (314,574), while (PF) 14.9 % (55,639) and mixed cases were 1.1% (4,300). In 2018 cases of provincial report showed that Sindh had highest number of malaria cases 34.5% (129,085), after which was Khyber Pakhtunkhwa 31.0% (115,995), Tribal Districts (merged districts) 17.6% (65,853), Baluchistan 16.4% (61,510), Punjab 0.5% (1,875) and then AJK 0.1% (195).[13]

This study aims at explaining the burden of malaria cases in Pakistan. Studies & surveys were carried out in different years to find the rate and spread of Plasmodium species and its spread with regards to environmental and seasonal changes.

MATERIAL AND METHODS

After getting the ethical approval from University of Lahore Pakistan, this study was carried out as community-based Cross-sectional study in endemic areas of all four provinces of Pakistan. The 2019 data related to Malaria from public and private facilities was utilized, and also census of 2017 population data was taken into account. Federally administered tribal areas of Khyber Pukhtunkhwa now known as the merged districts were excluded from the study because of the restrictions due to political instability and violence. [14]

Four most endemic cities were included in

this study from every province and the samples were collected from following
District Dir (Khyber Pakhtunkhwa province), District Mirpur khas (Sindh province), District Loralai (Balochistan province), District Jhang ((Punjab province), Samples were collected from all sites during the period of April-Oct 2020 which was considered to be peak malaria season. People of all ages with positive signs of malaria were included in research.
Blood was taken from patients who gave consent. Patient age and gender were also noted and then 03 ml of blood was taken from the vein and put into EDTA tube. 10% Giemsa solution was used for thick and thin films staining and examined at 100 x under oil immersion. The smears were examined according to WHO guidelines by trained technicians. [15]

RESULTS

A total of 450 microscopy-positive samples were done by PCR along with positive and negative controls. By PCR technique, 6.4% (29) were identified as P. falciparum, 85.8% (386) were P. vivax, and 7.8% (35) were mixed P. falciparum and P. vivax. Total 39 (8.7%) P. falciparum, 393 (87.3%) were P. vivax and mixed infections were 18 % positive in microscopy (Table 2). There were inconsistencies found among the PCR and microscopy results. PCR has shown precise results while identifying with exact number of patients with a particular specie of plasmodium like there were total 29 P. falciparum identified by PCR, rather than 39 P. falciparum specie identified by microscopy means 10 more patients were diagnosed with falciparum infection in the same way, 386 P. vivax were identified by PCR rather 393 were diagnosed microscopically means seven less patients were specified with P. vivax, and so for mixed infection number 35 were identified by PCR but only 18 were diagnosed on microscopy means 12 patients were more detected on PCR. Slides were not available for re-examination in the case of discrepancies between microscopy and PCR, but PCR was carefully done with all samples.
Of the 450 microscopy-positive samples, according to the latest data available the highest number of cases were in Sindh, next in Khyber Pakhtunkhwa then in Baluchistan and finally least cases were from Punjab. These numbers of cases were selected on the basis of data available in 2019 disease burden calculation in different provinces of Pakistan. The highest to lowest number of cases were already discussed in the above part. The results of the study showed that P. vivax was the most common malaria specie in study area while Falciparum stands second in line.

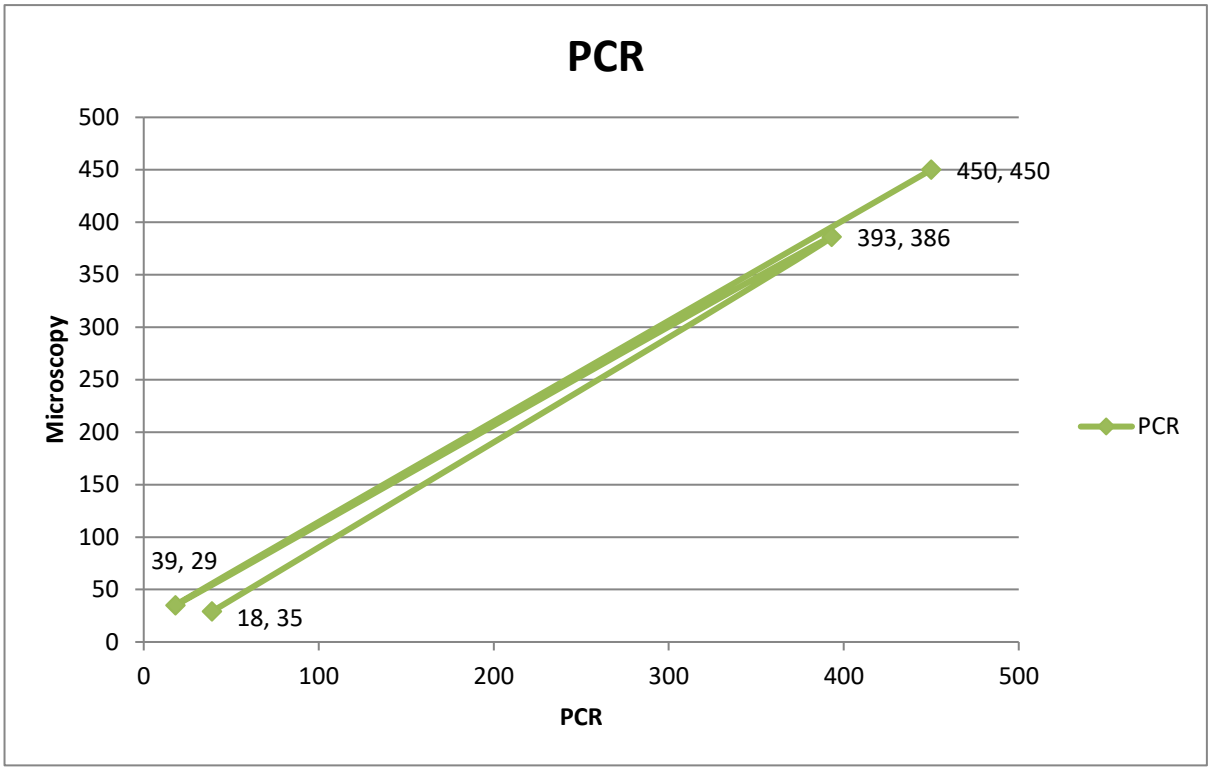
Table 1: PCR Positive tests for malaria.

	Frequency	Percent
Plasmodium Falciparum	29	6.4
Plasmodium Vivax	386	85.8
Mixed Infections	35	7.8
Total	450	100.0

Table 2: Microscopy Positive Lab test for malaria

	Frequency	Percent
Plasmodium Falciparum	39	8.7
Plasmodium Vivax	393	87.3
Mixed Infections	18	4.0
Total	450	100.0

Figure 3: Diagnostic accuracy of Microscopy verses PCR Technique



This above figure shows that among total 450 respondents microscopy results are very close to PCR, that is 386 vivax are identified by PCR and microscopy detected 393 vivax species positive in the same way 29 verse 39 falciparum specie and 35 verses 18 mixed infection were detected by PCR and microscopy respectively.

DISCUSSION

Among other serious public health problems malaria is still a major health hazard in Pakistani community. According to study reports of 2008, mortality from malaria was almost 50,000/year from a total 2.6 million reported malaria cases.[16] In 2010 EMRO reported over one million confirmed malaria cases out of which 22% were from Pakistan. [17] Exact estimation of prevalence of specie was difficult due to lack complete coverage and resources. The focus of this study was to get up-to-date information of malaria cases in highly endemic areas of Pakistan. Samples were collected from patients coming with symptoms, health seeking attitude and regional variation was a strong limitation. During the transmission season all patients were sampled coming to health facility but particular species are on peak during different months of the year. The month of April till September is peak season for P. vivax, whereas P. falciparum peak season is August till December.[18] These points were taken into account while assessing small reporting differences in the prevalence of malaria and in the proportions of both species among regions. In spite of limitations of the study this data contributed a lot in current condition of malaria in Pakistan.

According to results of previous studies the cases of malaria in males in their twenties were found to be higher as compared to females.[19] Some hypotheses for the unequal distribution of malaria disease among the males as compared to females is that the males are more exposed to Anopheles and they were more prone to be infected by the bite of mosquitoes. The study main finding depicts that malaria is highly prevalent in Sindh then Khyber Pakhtunkhwa followed by Baluchistan, with lowest prevalence in

Punjab. Few years back Punjab had highest number of malaria cases but with good case management techniques the number declined.

This study findings are consistent with the studies done before which claimed that the cases of P.vivax and Falciparum are the two major species prevalent in Pakistan, whereas P.vivax is more common as compared to falciparum.[20] According to studies done in past show the nearly quarter to one half of malaria cases were attributed to P. falciparum in these cities.[21] The discrepancies were found while detecting samples for microscopy-positive and PCR negative for Plasmodium, for samples that were P. falciparum or P. vivax mono infections by microscopy and mixed species infections by PCR. Repeated PCR test result showed that few error lies in diagnosis by microscopy. The major factors found to influence results accuracy of microscopy are use of polluted slides, faulty stains, artifacts, lack of experts in dealing manually and also the services provided. It has always been misdiagnosed that where P. vivax is high, P. falciparum must also be present in this patient, which will result in misinformation and false treatment.

CONCLUSION

Management and control of malaria is always a challenge in Pakistan as different tropical regions have different distribution of species, so lack of information poor skills for diagnosis lead to wrong diagnosis in Pakistan. This study results indicate that Sindh, Khyber Pakhtunkhwa and Baluchistan provinces should be the focus areas because prevalence of malaria is highest and in these endemic areas of Pakistan, proper laboratory facilities should be provided to improve species diagnosis while using microscopy technique in the areas where PCR facility is not available right now.

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AUTHOR'S CONTRIBUTION

SA: Research proposal development, Data collection, Analysis, Article writing and Reviewing
RA: Research proposal development, Analysis, Article writing and Reviewing
ZT: Research proposal development and Data collection

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Original Article

IS DOING A COMPLETE AUTOPSY AIDING IN REACHING THE CAUSE OF DEATH IN PAKISTAN?

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ABSTRACT

Background: One of the primary objectives of a forensic autopsy is to establish the cause of death. Various approaches may be taken to decide the extent of the autopsy required for a particular case. In Pakistan, all bodies found under suspicious circumstances are mandated by law to undergo a complete autopsy after registration of a first information report (FIR) by the police. This study aimed to compare and analyze the cause of death recorded in the police FIR before the autopsy is performed and the final autopsy report after a complete, 3-cavity autopsy has been done.

Materials and Methods: This retrospective study analyzed records of postmortem examinations carried out at a single, tertiary-level autopsy center between June 2009 to July 2018. The study included 769 reports of complete autopsies performed and compared the cause of death stated in the final autopsy report to the FIR recorded by the police.

Results: Of the 769 autopsies included, based on a comparison of the cause of death between police FIR and autopsy reports, five different groups were established. 504 (65.5%) cases were categorized as "similar", 74 (9.6%) cases as "dissimilar", 72 cases (9.4%) had no cause of death mentioned in the FIR, 65 cases (8.5%) had no cause of death mentioned in the autopsy report and 54 cases (7%) neither the FIR nor the autopsy report stated any cause of death. The leading cause of death noted to be similar in FIR and autopsy was from firearm injuries.

Conclusion: A large proportion of the cases included in the study had similar causes of death recorded in the FIR, documented before the autopsy was done and the autopsy report. The value of doing a complete autopsy stands questioned and it may be advisable to look into adopting alternate practices as seen in other parts of the world. Moreover, outdated, or absent guidelines for the actual carrying out of autopsies must be looked into and policies/ legislations surrounding it reviewed.

Key Words: Autopsy, Cause of death

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INTRODUCTION

A postmortem examination or a medico-legal autopsy is an examination of the dead body by the statutory mandated medico-legal examiner to provide a neutral and objective medical assessment, primarily of the cause and manner of death and to establish the identity, if unknown.[1] The purpose of the forensic autopsy is to assist the legal justice system at the request of law enforcement agencies about deaths under unexpected, dubious, inexplicable, abnormal, disputed, or suspected nefarious circumstances.[2] The autopsy performed may include a mere external examination, encompassing a partial autopsy, or a complete autopsy in which all the body

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cavities are mandatorily opened, and all specified samples collected. [3,4]

An autopsy commences with identification of the body with comments on the size, build and state of health.[5] This is followed by an exploration into the mode (respiratory, cerebral, or cardiovascular failure) and time of death to lead to the ascertainment of the cause of death. Internal and external examinations are carried out and any peculiarity or injury is noted. Samples for microbiological, histological, and toxicological examinations are obtained to confirm or refute the physical findings.[6] The entire examination and all the findings, positive or negative are recorded in a clear and systematic format on a proforma for reference.[3] Beyond this point the procedural commonality of a forensic autopsy ends.

The approach taken to a forensic autopsy varies greatly across different regions, that is, the process followed to tag a body for a forensic autopsy and whether this will be a complete or partial autopsy as is required for a particular case. In England, Wales, and Australia, under the Coronial system, a notifiable death is referred to the coroner, who may either be a qualified solicitor or a Fellow of the Chartered Institute of Legal Executives and must have more than 5 years of experience. The coroner then decides the extent (complete or partial) of the autopsy which will take place.[3] In Scotland, under the non-invasive "view and grant" system, an experienced pathologist externally examines the unclothed body of the deceased taking into consideration the deceased's history and the events surrounding the death. The pathologist will then confirm to the Procurator Fiscal (Scottish counterpart of a coroner) that a full postmortem examination is required or not based on their position to certify the cause of death following the "view and grant" examination .[7] This system aims at ascertaining the most plausible cause of death while maximizing on expertise and minimizing on resources and time.

In Pakistan, forensic autopsy is mandatory for every reported, unnatural death under article 174 C of the Code of Criminal Procedure Pakistan (CrPC). Unnatural deaths can be reported by the police, next of kin or the attending doctors. The autopsy may be ordered by the superintendent of police or a magistrate.[8] It is noteworthy that neither the SP nor the magistrate has any training in forensic pathology nor is their decision based on any discriminatory evidence. The law mandates that every suspicious death undergo a complete autopsy which includes external and internal examinations with opening of all three cavities and sample collection for analysis. The body tagged for autopsy is handed over by the police to the autopsy center. The documents required along with the body include the police's First Information Report (FIR), containing the presumed date, time, apparent cause, and circumstances surrounding the death. The autopsy surgeon carries out an autopsy for every case, filling out prescribed proforma and sending samples collected to the forensic lab for further analysis.[9]

Australia, England & Wales	Scotland	Pakistan
↘	↓	↙
Body found under suspicious circumstances/ notifiable death		
↙	↓	↘
The coroner orders an autopsy if needed	A trained pathologist advises Procurator Fiscal if an autopsy needed	SP or Magistrate order autopsy of all bodies
↓	↓	↓
Autopsy ordered	Autopsy ordered	Autopsy ordered
↙↘	↙↘	↓
Yes No	Yes No	Yes
↙↘	↙↘	↓
Complete Partial	Complete Partial	Complete

Figure-1. Comparative flow chart of approaches taken to autopsy in Australia, England, Scotland, Wales, and Pakistan.

The approach to autopsy and its consequential contribution to the establishment of the cause of death warrants a study of the current practice in Pakistan. In this study, the objective is to ascertain whether the current practice of carrying out a complete autopsy for every case helps in establishing the cause of death. To the best of our knowledge, no similar work has been done in Pakistan regarding this topic. In this study, autopsy records available of the last 10 years of a single, tertiary-level autopsy center were obtained and the cause of death as recorded in the police's first information report were compared and analyzed with the final autopsy report.

MATERIAL AND METHODS

The study is a retrospective, descriptive study including 769 autopsy reports entered into the autopsy registers at one of the main autopsy centers in Lahore, Pakistan (the name of the autopsy center being kept confidential due to the sensitive nature of data involved). The reports included were those which could be obtained, and which were marked as completed. Permission was obtained from the competent authority of the autopsy center and ethical approval was obtained from the Ethics Review Board of Continental Medical College, Lahore, Pakistan. The reports included in the study were based on non-probability, convenience sampling of autopsy examinations performed from July 2009 to July 2018. The reports included autopsies performed on bodies brought by the police along with the FIR for each case. The data from the police FIR and the final autopsy report by the autopsy surgeon for each autopsy were tagged by the autopsy number issued by the autopsy center. Then data from each autopsy was entered into MS Excel sheets. The causes of death in all the reports were analyzed and were classified into 25 main categories. This was done to minimize the variation in terminology used and for standardization of categories formed. For comparison, the number of deaths classified into a specific category (of cause of death) based on the FIR and by the autopsy were entered into two separate columns. Where the causes of death were the same in both columns, it was marked as "similar". Where the cause of death mentioned in the FIR was different from the autopsy report, it was grouped as "dissimilar". A "no comment" group was made for cases where neither the FIR nor the autopsy report stated a cause of death. The final two groups were "no comment by police" and "no comment after autopsy" where the police or the autopsy report were silent respectively. The data was analyzed using percentages of the number of cases in each group.

RESULTS

The total number of cases included in the study was 769, which were categorized into 5 groups. The groups were based on a comparison of cause of death as per FIR and autopsy. Table 1, mentions the number and frequency of cases in each group.

Table-1. Groups based on comparison of cause of death between FIR and autopsy report, along with respective number of cases and frequencies

Sr. No.	Groups	Number of cases (n= 769)	Percentage of cases (%)
1	Similar	504	65.5
2	Dissimilar	74	9.6
3	No comment by the police	72	9.4
4	No comment after the autopsy	65	8.5
5	No comment	54	7

The highest number of cases were noted in the similar group indicating that in 65.5% of cases, FIR and autopsy findings were categorized under the same cause of death. Two groups were identified in which there were no comments on the cause of death based on the autopsy, with the aggregate frequency of these two groups being 15.5%. Furthermore, two groups were identified in which the FIR-based cause of death was absent, the total frequency of these groups being 16.4%.

Expansion of the data in the similar group is shown in Figure 1. This group included 504 cases with the highest cause of death being firearm injury, amounting to 242 cases. Firearm injuries constituted 48% of the cause of death in the similar group and 31.5% of all deaths. This was followed by road traffic accidents and sharp weapon injuries forming the second largest cause of death at 8.1% and 7.9% respectively in the "similar" group and 5.3% and 5.2% respectively of all the cases. Altogether, 323 cases out of 769 (42%) were noted to have the same three causes of death before and after a complete autopsy was performed, comprising of firearm injury, road traffic accident and sharp weapon injury.

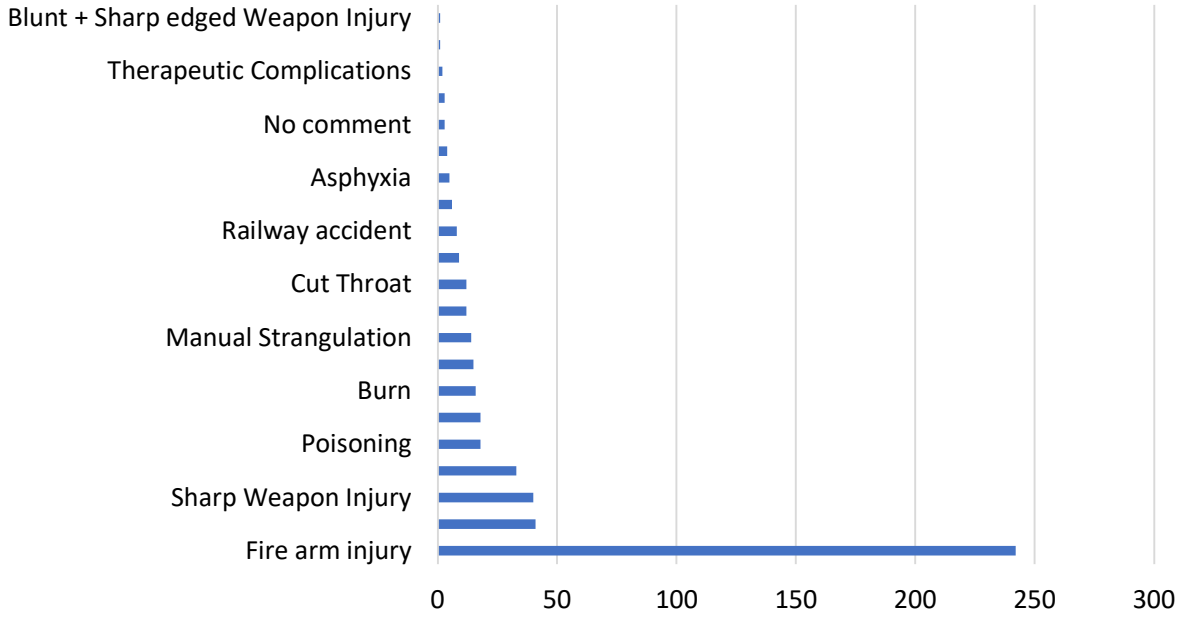

Figure-1. Number of cases belonging to different cause of death in "similar" group.

DISCUSSION

Death perhaps is the only certainty of this world. Matters beyond death would be dabbling in religion or philosophy. But death and the manner of death, more specifically one considered unnatural, is subjected to forensic examination and inquiry into its causes forms the basis of investigations helpful to the legal justice system. The results of this study show that in 65.5% of cases, the autopsy yielded similar results for the cause of death as the police report, the latter being based on details surrounding the death, evidence found and interviews of witnesses and families. This reveals that in the majority of the cases, the cause of death remained unchanged after a complete autopsy was performed. Therefore, it may be inferred that the actual need to do a complete autopsy existed for 34.6% of the cases only. Davidson.[7] reported that only 41% of deaths required an internal autopsy under the procurator fiscal using the "view and grant" procedure in Scotland. The findings in our study may be comparable to Scottish standards given that if alternative approaches had been adopted, the requirement for complete internal autopsy could have been limited to 34.6% (well under 41%) of the cases as opposed to 65.5%. In the US, individual states have different laws about the approach taken for an autopsy to be performed. However, complete autopsies are seldom mandated since a complete external examination along with toxicology may suffice to elucidate the cause and manner of death. [10] In the Kingdom of Saudi Arabia, complete autopsies were carried out on only 31.3% of medico-legal cases reported in 2007, while 68.7% of cause and manner of death certificates were issued based on external examinations only. [11] The statistics seen in our study raise questions regarding the necessity of the number of complete autopsies being performed and how different approaches, such as the "view and grant" may be feasible alternate approaches. A scrutiny of the group of similar results reveals that the highest degree of concordance in the cause of death between the FIR and the autopsy report was for firearm injury (48%). Based on the results of the present study, the establishment of the cause of death due to firearm injuries gains nothing from the performance of a complete autopsy. Reasons for performing an autopsy in these cases may be to assist in other aspects of the investigation, for instance, identification of the body or establishment of manner of death. However, a complete autopsy mandates that all three cavities, skull, chest, and abdomen are opened. When the cause of death is not a question, the rationale for a complete autopsy stands challenged. The second most frequent cause of death noted in the "similar" group is due to road traffic accidents. Similar findings were noted in a study conducted in Australia which revealed that in cases of deaths from motor vehicle accidents, complete internal autopsies added nothing to the cause of death beyond the obvious cause of succumbing to injuries due to the motor vehicle accident. [3] Cumulatively the top four causes of death in the similar group formed 70.6% of all cases in this group. This highlights the fact that alternative approaches can help in bringing down the number of redundant autopsies in terms of establishing the cause of death beyond the obvious which was elicited before the performance of the complete autopsy. Moreover, a complete autopsy is a resource-intensive procedure in terms of finances as well as time taken to perform one. This is burdensome for far more affluent and developed systems and an enormous challenge for a developing nation like Pakistan. According to a study in England, trained forensic pathologists were asked to base predictions about the cause of death using a paper exercise and without an actual examination of the body, which when verified by autopsy later, were found to be correct in 39% to 46% of cases.[12] Another study from Australia revealed that the presumed cause of death determined by

the medical examiners and experienced pathologists without performing an autopsy was completely wrong in 28% of cases. [10] Comparing the results of these studies involving experienced and trained pathologists with the Pakistani police's initial cause of death being right reveals that their opinion about the cause of death was correct in 65.5% of cases as echoed by the autopsy results. Furthermore, in 8.5 % of cases, the police opinion was noted to be the only opinion about the cause of death despite the performance of a complete autopsy. This cumulatively makes the police's conjecture on the cause of death correct in 74% of cases, which is the highest correct number in comparison to the English and Australian studies mentioned above. Crime scene handling and forensic investigations by the police require specialized training which is not a part of regular police training or work in Pakistan. Therefore, the results of the present study unrealistically indicate exceptional proficiency by the Pakistani police or paradoxically it may serve to highlight the inadequacy of the autopsy being performed. Despite the performance of a three-cavity opened, complete autopsy, in 15.5% of cases the autopsy failed to establish any cause of death (notwithstanding that the police have proffered an opinion in 8.5% of these very cases). It must be pointed out that in Pakistan, the autopsy surgeon is required by law to be a doctor with no further mandatory specialized training in forensics or forensic pathology.[13] A ruling by the Lahore High Court underscores that the autopsy-performing doctors lack adequate training. Carrying this forward may help to explain why the autopsy has identical results in 65.5% of cases, as the autopsy surgeon has no independent finding to add regarding the cause of death. In 8.5% of cases, the autopsy yielded nothing where the FIR reported nothing and the most disquieting of all these figures is the 7% of cases in which despite a complete autopsy, nothing was stated regarding the cause of death. These statistics make one wonder about the competence of the autopsy surgeons to perform an autopsy. Finally, confounding matters further is the lack of any clear standard operating procedures or guidelines for the performance of an autopsy. This was brought to the forefront in an order of the Lahore High-Court Multan Bench. [14] in which the reference for autopsy guidelines are from a textbook of forensic medicine and toxicology rather than guidelines issued by the competent medical and legal authorities of the country, which would be adapted to local challenges and regulated by local laws, as seen in other countries such as guidelines issued by Royal College of Pathology in case of the United Kingdom. [15] Smith.[16] notes that in 80% of cases in England, cause of death can be ascertained based on basic investigation and does not require autopsies. Furthermore, Carpenter and Tait.[3] point out in their review of the Coronial system, that there is an over-ordering and over-reliance on autopsies which could be minimized based on a detailed examination of the circumstances surrounding the death and coroners with a clearer understanding of medico-legal aspects. Pakistan is a predominantly Muslim country where the majority believe that an autopsy is considered mutilation of the body. [17] coupled with scarce resources, innumerable, unnecessary autopsies as mandated by the current law may conversely be slowing the process of justice by failing to achieve their objective. Analysis of the data indicates that the balance of reason for performing a complete internal autopsy must therefore tilt heavily towards circumstantial evidence pointing to its need. Moreover, an audit of the current data may aid in the development of protocols and procedures aimed at improving the efficiency of the current system. It may also be noted that current legislations are outdated and require revision. And finally, the competence of the autopsy surgeon must be impeccable with well-defined targets and responsibilities. Some of the limitations encountered were

that the data used in this study was from a single center and there may be variations in data from other centers. Furthermore, the reports included were not an exhaustive record of the duration from which data was collected and were only those to which the researchers were given access. Furthermore, despite the high standards maintained in the English system, which aims to maximize efficiency, accuracy, economy, and timeliness without compromising on integrity of the death investigation. Moreover, based on the results of our study, the actual need for performing complete autopsies may be similar to those reported by Smith. [16]

CONCLUSION

Analyzing the degree of concordance between the FIR-based cause of death with the final cause of death as ascertained on autopsy leads one to question the need to perform complete autopsies and the laws about ordering one. Borrowing from various, robust systems existing globally, the percentage of complete autopsies could be reduced to ensure that only those cases are processed for a complete, three-cavity-opened autopsy which truly warrants one. Moreover, the existence or the lack thereof of adequately trained professionals, standard protocols, and updated legislation for carrying out autopsies needs to be looked into to ensure rationalization of the autopsies being carried out, especially about determining the cause of death.

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AUTHOR'S CONTRIBUTION

AZ: Research proposal development, Data collection, Analysis, Article writing and Reviewing
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AM: Analysis, Article writing and reviewing.

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Origional Article

Functional bowel disorders after COVID-19

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ABSTRACT

Background: To assess gut-brain interaction disorders and gastrointestinal symptoms after COVID-19 hospitalisation.

Material and Methods: This prospective study was done on Medical wards and ICUs of Jinnah Hospital Lahore, Mayo Hospital Lahore, DHQ Hospital Gujranwalla, and Hijaz Hospital Lahore from April 1, 2020–December 31, 2021.We enrolled 1284 patients (both COVID-19 and non-COVID-19) who met the inclusion criteria and followed them for 1, 6, and 12 months after hospital discharge. Validated questionnaires measured depression, anxiety, and gastrointestinal symptoms. 881 patients were included in the primary analysis after excluding those with preexisting GI symptoms or surgery. (270 controls, 611 COVID-19).

Results: Out of 805 (62.7%) of the remaining 1035 had COVID-19, and 162 (94 COVID-19 and 68 control) were excluded due to history of existing gastrointestinal symptoms or surgery in the past. 873 subjects without pre-existing confounders were assessed and followed up for primary and secondary aim analysis. 746 patients completed 6-month and 603 patients completed 12-month follow-up evaluations. In primary aim analysis, mean age was 48.9 ±20.1 years for control group and 52.9±14.2 for COVID patients (p=0.47). 62.1% of control and 58.7% of COVID cases were male (p=0.54). BMI in control group was 24.8±7.5 and in COVID cases it was 23.9±7.6 (p=0.6). COVID-19 patients had more gastrointestinal symptoms at enrollment (65.5% vs 38.5%, p<0.0015). Controls (15.5%) have more constipation than COVID-19 patients (9.1%) at 12 months (p=0.029). ROME IV-defined IBS was higher in COVID-19 patients (4.0% vs. 0.3%, p=0.035). IBS was linked to allergies, dyspnoea, and proton pump inhibitors.At 6 months, the rate of depression among COVID-19 patients was higher than that of controls.

Conclusion: At 12 months, patients hospitalised with COVID-19 had less number of hard stools and constipation than controls. COVID-19 patients had significantly more IBS than controls.

Keywords: COVID-19, Nasopharyngeal swab, ROME IV, Irritable Bow syndrome.

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INTRODUCTION

In June 2022, the WHO reported over 5 million COVID-19 cases and over 6 million deaths worldwide. There is a wide range of possible symptoms associated with COVID-19, from asymptomatic to potentially fatal.[1] Elderly and sick people are more likely to get serious illnesses. New virus variants cause cyclical peaks despite vaccination.[2]

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The newly emerging COVID-19', which is characterised by symptoms like dyspnea, fatigue, arthralgia, cognitive disturbances, chest pain and compromised quality of life after SARS-CoV-2 infection, is straining healthcare systems worldwide. [2,3] A meta-analysis of 57 studies, found long-term sequelae of respiratory impairment, neuropsychiatric disorders, functional impairments and presence of constitutional symptoms in COVID-19 survivors. Abdominal pain, diarrhoea, anorexia, nausea and vomiting were long-term digestive symptoms.[4,5] The background mechanism of these symptoms includes virus-induced prothrombotic state, gut dysbiosis, cellular injury and enteric nervous system dysfunction [6-9] In addition, these so-called "long COVID-19 symptoms" may be similar to post-infection (PI) disorders of the interaction between the gut and the brain (DGBI). Post-infectious IBS is most strongly linked with acute gastroenteritis caused by bacterial or viral pathogens (PI-IBS).[10] Fewer studies have looked at IBS and other DGBI caused by viruses than have looked at those caused by bacteria. Due to limited follow-up, small sample size, lack of controls, and retrospective study design, the long-term effects of these disorders are unknown.[11] The objective of this study is to compare hospitalised patients with COVID-19 to non-COVID to see which group had a higher prevalence of gastrointestinal symptoms.

MATERIAL AND METHODS

In this prospective study in which 1284 patients of both genders, aged between 15 to 80 years hospitalised with or without COVID-19 (WHO-defined COVID-19 diagnosis –Positive Nasopharyngeal swab for COVID-19 PCR), were enrolled upon admission from April 2020 to December 2021 and reassessed for their symptoms at 1, 6, and 12 months. All patients gave written consent and were evaluated according to clinical symptoms. Patients with concurrent cancer or mechanical ventilation were excluded. The control group included patients from Jinnah, Mayo, and Hijaz hospitals' internal medicine units and ICUs who were hospitalised for reasons other than COVID-19. Demographic characteristics, medical history, laboratory data, and the Gastrointestinal Symptoms Rating Scale (GSRS) were recorded at admission and follow-up(1, 6, and 12 months).

GSRS is a 1-week recall tool for IBS and peptic ulcer disease that grades 15 common gastrointestinal symptoms on a 7-point scale. It was used to assess patients for COVID-19-related-gastrointestinal symptoms. Patients who had symptoms within the previous six months of hospitalization were also screened out using the GSRS. These patients were called and interviewed at follow-up to calculate GSRS and Hospital Anxiety and Depression Scale (HADS). [12] Depression and anxiety are rated as follows by the HADS: 0–7, normal; 8–10, borderline abnormal; 11–21, abnormal. The Rome IV Diagnostic Questionnaire for Functional Gastrointestinal Disorders in Adults was used to diagnose DGBI at 6 and 12 months.[13]

The primary endpoints were DGBI and long-term gastrointestinal symptoms after COVID-19. After finding a statistically significant difference between groups, the secondary endpoints assessed predictive factors of PI DGBI. Exploratory endpoints included DGBI, long-term gastrointestinal symptoms, anxiety, and depression at the 12-month follow-up.

Means and standard deviations were reported for quantitative data, while frequencies and percentages were used to describe qualitative data. Primary and secondary aim analyses were performed after excluding patients with prior gastrointestinal symptoms or recent surgery. Prior gastrointestinal symptoms

included at least two GSRS items starting at least six months before hospitalisation. COVID-19-negative patients made up the control group. Admission and follow-up data were compared using the Mann-Whitney U test, Fisher's test and Student's t-test. At a follow-up of 12 months,

Histograms described the data about DGBI, anxiety, and depression and logistic regression analysis (univariate and multivariate) predicted the occurrence of GSRS and DGBI. We estimated the Odds ratio, 95% Confidence interval, and p values. SPSS 24 performed all analyses.

RESULTS

1284 hospitalised patients were consecutively enrolled from April 01, 2020, to December 2022. 249 patients were excluded because they did not follow the study protocol (died), had missing questionnaire data, or were diagnosed with cancer during follow-up. 805 (62.7%) of the remaining 1035 had COVID-19, and 162 (94 COVID-19 and 68 control) were excluded due to prior gastrointestinal symptoms or recent surgery. 873 subjects without pre-existing confounders were evaluated and followed up for primary as well as secondary study goals. 746 patients completed 6-month and 603 patients completed 12-month follow-up evaluations. At enrollment, COVID-19 patients had a higher rate of gastrointestinal symptoms compared to control (59.3% vs 39.7%), $p<0.001$(Table 2). They had higher rates of nausea, diarrhoea, loose stools and urgency. At 1 month, COVID-19 patients had high rates of acid regurgitation and nausea. At the 6 and 12-month follow-up, these patients had low rates of hard stools and constipation compared to the control group. No other GSRS results differed between groups.

At the 6-month follow-up, both groups had similar rates of epigastric pain, post-prandial distress syndrome, functional dyspepsia, IBS and functional diarrhoea (table 2) but COVID-19 patients had a higher depression rate than controls: borderline abnormal, 9.9% versus 4%, and abnormal, 4.2% versus 2.7% ($p=0.014$). COVID-19 patients had significantly higher IBS (4% vs 0.3%, $p=0.035$) (figure 2, table 2) and other DGBI rates than controls at 12 months. Post-COVID IBS patients had significantly higher baseline rates of antibiotic intake, cough, dyspnoea, and headache during hospitalisation in the previous 3 months. In post-hoc analysis, clinical and demographic data at baseline, 6-months, and 12-months were tested as independent predictors of IBS diagnosis for the study cohort selected for primary aim evaluations. COVID-19, allergies, and PPI use predicted IBS in the post-hoc analysis of the entire study cohort. Univariate analysis showed that comorbidities, cough and dyspnea at enrollment, PPI intake, antibiotic intake within three months before hospital admission, anxiety at the six-month follow-up, and in-hospital antibiotic administration were predictive factors for IBS in COVID-19 patients (table 3). Three variables were found significant in multivariate analysis: allergies, chronic PPI use, and dyspnoea.Figure 4 shows a nomogram of risk factors for IBS at 12 months.

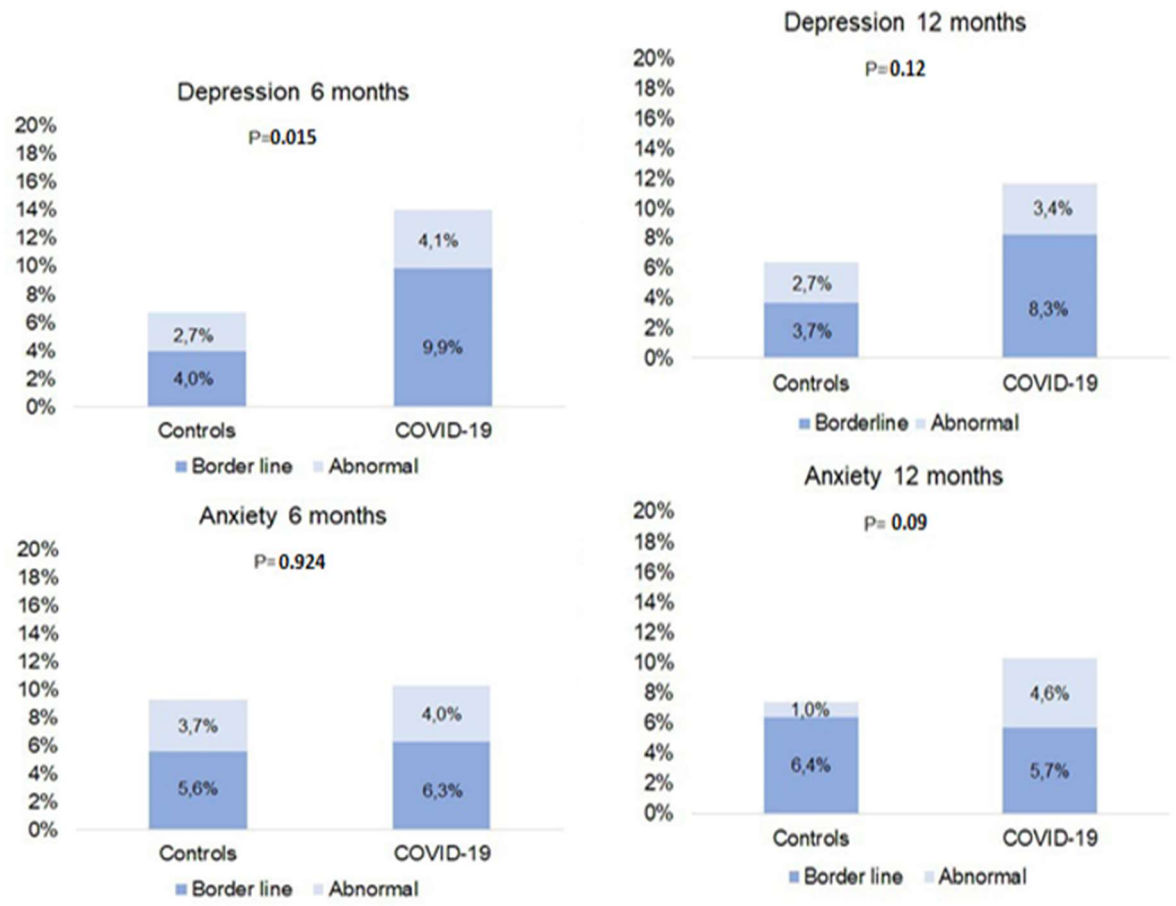

Figure 1: HADS measurements done in follow up period in patients with COVID-19 and controls.

Table 1: Demographics of patients in primary aim analysis in the study

	Control group, **n (%) or Mean±SD n= *264***	COVID-19, **n (%) or Mean±SD n=609**	**P value**
Age	48.9±20.1	52.9±14.2	0.47
Sex, male	159 (62.1)	358(58.7)	0.54
BMI	24.8±7.5	23.9±7.6	0.06
Smoker			0.001
Current	72 (27.3)	59 (9.8)	
Former	66 (25)	112 (18.4)	
Physical exercise (at least 45 minutes three times per week)	90 (34)	152 (24.9)	0.88
Comorbidities			
Neuropsychiatric	30 (11.4)	22 (3.6)	<0.01
Cardiovascular	105 (40)	174 (28.6)	0.001
Pulmonary	31 (11.7)	41 (6.7)	0.013
Hepatic	16 (6)	20 (3.3)	0.046
Renal	20 (7.6)	28 (4.59)	0.082
Diabetes	60 (22.7)	88 (14.4)	0.004
Allergies	13 (4.9)	18 (2.95)	0.157

Autoimmune	11 (4.2)	17 (2.8)	0.302
Blood disorders	11 (4.2)	8 (1.13)	0.011
Chronic medication intake with GI effect			
PPI	69 (26.1)	75 (12.3)	0.002
NSAIDs	33 (12.5)	33 (5.4)	0.001
Steroids	13 (4.9)	7 (1.1)	0.002
Metformin	16 (6)	31 (5.1)	0.585
SSRI	9 (3.4)	11 (1.8)	0.154
Antipsychotic	4 (1.5)	3 (0.5)	0.126
Thyroxine	11 (4)	19 (3.1)	0.454
Rifaximin	3 (1)	0	0.008
5-ASA	3 (1)	5 (0.8)	0.665
Probiotics in the last 3 months	28 (10.6)	46 (7.5)	0.126
Antibiotics in the last 3 months	91 (34.6)	132 (21.5)	<0.001

Table 2: Prevalence of anxiety, DGBI, and depression at the follow-ups among patients who met the study's eligibility criteria for the primary aim analysis

	Follow up at 6-Month			***Follow-up at 12 month***		
	Control group **n (%) n=*211***	COVID-19 **n (%) n=*535***	**P value**	Control group **n (%) n=*178***	COVID -19 **n (%) n=*425***	**P value**
Anxiety			0.92			0.098
Borderline abnormal	13 (6.16)	30 (5.6%)		13 (7.3%)	26 (6.1%)	
Abnormal	7 (3.31)	19 (3.6%)		1 (0.56%)	19 (4.4%)	
DGBI						
Functional dyspepsia	4 (1.9)	11 (2)	0.53	4 (2.1)	16 (3.7)	0.31
Postprandial distress syndrome	4 (1.9)	10 (1.9)	0.76	4 (2.1)	18 (4.2)	0.13
Epigastric pain	1(0.4)	4 (0.74)	0.25	3 (1.9)	9 (2.1)	0.48
Chronic nausea and vomiting syndrome	3 (1.3)	6 (1.1)	0.77	3 (1.9)	2 (0.5)	0.14
Functional diarrhoea	0	1 (0.2)	0.52	0	1 (0.2)	0.51
Cyclic vomiting syndrome	1 (0.5)	0	0.12	—	—	—
IBS	2 (0.94)	3 (0.56)	0.58	1 (0.3)	17 (4%)	0.035
HADS						
Depression				0.0150.1		
Borderline abnormal	10 (4.7)	55 (10.3)		8 (4.5)	35 (8.2)	
Abnormal	6 (2.8)	22 (4.1)		4 (2.3)	16 (3.7)	

Table 3: Logistic regression analysis to identify factors associated with occurrence of IBS at 12 months follow-up in COVID-19 patients in the primary aim analysis group.

	Univariate	P value	Multivariate	P value
	Odd ratio (95% CI)		Odd ratio (95% CI)	
Clinical course				
Dyspnoea	4.167 (1.369 to 12.680)	0.012	4.157 (1.336 to 12.934)	0.013
Cough	4.935 (1.091 to 22.321)	0.038		
Anxiety (at 6 months according to HADS)	2.091 (0.994 to 4.357)	0.052		
Antibiotic administration during				
Hospitalization	3.975 (0.861 to 16.951)	0.076		
Coexisting conditions/Comorbidities				
Allergies	6.221 (1.229 to 32.148)	0.025	10.123 (1.765 to 56.881)	0.008
Hepatic diseases	4.846 (0.989 to 23.734)	0.051		
Chronic medication intake				
PPI	4.031 (1.301 to 11.49)	0.017	4.826 (1.457 to 17.026)	0.011
Antibiotic use in the last 3 months	3.168 (1.061 to 9.320)	0.036		

DISCUSSION

This prospective study compared the cohort of hospitalised COVID-19 patients to a control group of non-COVID patients. After hospitalisation, patients were followed for 12 months and adjusted for previous gastrointestinal symptoms, surgery, and medication use. This study revealed that the majority of gastrointestinal symptoms improved after being hospitalised for primary disease. These patients had more IBS and less constipation/hard stool than controls at 12 months but did not differ from control in GSRS domains. These findings contradict previous reports. COVID-19 survivors had more loose stools and no constipation at 6 months, according to one of the studies. [14] These patients are more likely to use laxatives and have constipation, according to large retrospective matched-controlled studies.[15,16] The COVID-19 group had a higher incidence of IBS (4%) than the control group, but it was lower than previously reported, which is up to 16% according to Rome IV criteria at 6 months .[16, 21] Our stringent patient selection may explain these discrepancies. Another study found an IBS rate of 6.4%, which is very close to our post-COVID-19 rate.[17] Post-COVID-19 GI symptoms have been studied in several previous studies but these studies have limitations due to small sample size, short follow-up time and retrospective and cross-sectional design. They had not deployed Rome IV criteria and had not adjusted analyses for the pre-COVID gastrointestinal symptoms. Additionally, they have not omitted the confounders of study [18] The risk of IBS in COVID-19 patients is higher if they have a history of allergies, dyspnoea and chronic PPI use. These patients had higher levels of depression and anxiety at followup. Our findings supported the link between post-COVID-19 IBS and allergies, immune dysregulation, and mucosal homeostasis.[19] The presence of dyspnea at baseline is also predictive of IBS following COVID-19. COVID severity may cause chronic intestinal symptoms.[20] PPIs may alter gut microbiota and this may cause post-COVID-19 gastrointestinal symptoms COVID-19 patients have reduced

microbial diversity, greater colonization of Bacteroides and substantially reduced butyrate-producing bacteria.[21] Alteration in enteroendocrine cell function, gut motility and permeability, and serotonin metabolism may be involved in microbiota changes and cause the persistence of symptoms. This viral infection can infect the ileum and colon due to more affinity with ACE2 receptor, thus it may cause de novo IBS (as viral gastroenteritis outbreaks cause IBS). Amplification of viral nucleic acids and immune activation have been found in the ileum of these patients up to six months after acute infection. Other studies have found an augmentation of the cytotoxic T-cell number in these patients.[22] In contrast to other PI-IBS, which typically peak soon after the acute infection, the peak of post-COVID-19 IBS occurs 6- 12 months later.[23] There are a few caveats to our study. First, there may be some other confounding factors that were not considered here. Additionally, the 12-month follow-up and use of GSRS to assess pre-hospitalization symptoms also affected our results as they might introduce a recall bias and have affected the outcomes. Second, we might not have been able to detect statistically significant DGBI in the COVID-19 patients as we excluded subjects who had pre-hospitalization gastrointestinal symptoms, thereby increasing the likelihood of type II error. In addition, there are some random drop-outs at each time-point, but this may not have affected our endpoints. Third, the control group of hospitalized patients had more comorbidities and medications at baseline than COVID-19 patients, which may have affected our results. Our study suffered overfitting variable bias due to a very low number of IBS patients. We selected variables based on pathophysiological reasons to partially overcome this limitation. Due to hospital access restrictions, patients were interviewed by phone at follow-up, potentially introducing questionnaire bias. Finally, our study only included inpatients from only three hospitals; this means that our results may not apply to the general public or to those who are not hospitalized. In conclusion, COVID-19 is linked to an increase in chronic gastrointestinal symptoms and IBS. Given the global distribution of disease, new-onset gut-brain interaction disorders are expected. To understand the mechanisms of these symptoms and find new remedies, more research is neede.

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Origional Article

ANTIOXIDANT ROLE OF MENTHA (MINT) AGAINST CHLOROQUINE-INDUCED OXIDATIVE STRESS IN MALE ALBINO MICE

Sumbal Khalid[1]

ABSTRACT

Background: Many drugs, in the world, have been found to induce oxidative stress when these are given at larger doses or for longer periods. Worldwide research work is being carried out to find the antioxidant role of many herbs and plants so that these can be given prophylactically to prevent oxidative damage, done by free radicals. The objective of the study was to determine the antioxidant role of mint against chloroquine induced oxidative stress.

Material and Methods: In this randomised controlled study, ninety male albino mice were divided into three groups randomly. Each of the three groups contained 30 mice. Group A was labelled as a control group and Group B and C were labelled as experimental groups. Chloroquine (970 mg/kg of body weight) was given orally to the mice of group B, on the 9th day of the experiment. The mice of group C were given an ethanolic extract of mint consecutively for the initial eight days then they were given chloroquine, at the dose of 970 mg/kg of body weight, on the ninth day. The ethanolic extract of mint was then continued to be given from day 10 to day 16 of the experiment. Blood samples of the mice were obtained on the 17th day of experiment by intracardiac puncture technique. SPSS version 20 was used to analyze the data.

Results: A highly significant (p=0.000) decrease in serum glutathione peroxidase and a highly significant (p=0.000) increase in serum malondialdehyde was observed in mice of group B (whom chloroquine was given) as compared to those of group A. Group C mice, to whom ethanolic extract of mint was given before and after the administration of chloroquine, showed a highly significant decrease and highly significant increase in serum levels of malondialdehyde and glutathione peroxidase respectively.

Conclusion: Chloroquine, when given at a dose higher than the therapeutic dose in mice, can induce oxidative stress. Mint has antioxidant potential against chloroquine induced oxidative stress.

Key Words: Chloroquine, mentha, oxidative stress, antioxidant.

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INTRODUCTION

Drugs, when given at higher doses or for longer duration, can generate free radicals, which can induce oxidative stress. Hence, the drugs are responsible for many side effects due to their oxidative potential.

For example, it has been reported that 50 % of liver failure cases in the world occur due to drug-induced hepatotoxicity.[1] It is estimated that more than 1000 drugs are responsible for acute liver failure.[2]

Previous research has revealed that hepatotoxicity is induced by various drugs due to oxidative stress induction.[3]

The radicals, generated by the drugs, can attack the lipids of the cell membranes.[4] When polyunsaturated fatty acids are degraded, certain secondary metabolites are formed, such as malondialdehyde.[5] The raised serum levels of malondialdehyde are indicative of oxidative stress induction.[6] Chloroquine is also one of the drugs which has the potential to induce oxidative stress if a dose higher than the therapeutic dose is taken.[7] Chloroquine, if given at a dose higher than the therapeutic dose, can induce hepatotoxicity due to the

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induction of oxidative stress.[8] The enzymatic and non-enzymatic antioxidants, in the body, prevent oxidative stress.[9] These antioxidants can scavenge free radicals. Excess formation of free radicals leads to excessive utilisation of antioxidants.[10] As a result, in oxidative stress, the serum levels of antioxidants are markedly reduced.[11]

Researchers have been going around the world to find different herbs and plants which have antioxidant potential.[12] Mint (mentha) is one such plant, which is being studied for its antioxidant potential.[13] It has been found that mint is a rich source of antioxidants.[14] Flavonoids and polyphenols are present in abundance in the mint. These antioxidants activate and make new antioxidant enzymes in the body. These antioxidants also scavenge free radicals. 25 different species of mint have been discovered in the world. In Pakistan, mentha arvensis is one of the most widely used species.

The aim of this study is to access antioxidant role of mentha (mint) against chloroquine-induced oxidative stress in male albino mice.

MATERIAL AND METHODS

Ninety male albino mice were included in this randomized controlled study. The mice were bought from the University of Veterinary and Animal Sciences, Lahore. Mice were selected by non-probability consecutive sampling method. Mice were then divided into three groups randomly, with each group containing 30 mice. Group A was labelled as a control group. Group B was the experimental group, in which each mouse was given chloroquine orally, at the dose of 970 mg/kg of body weight on the 9th day of the experiment. Group C was also an experimental group in which ethanolic extract of the mint was given from day 1 of the experiment to day 8 of the experiment. Then, chloroquine at the dose of 970 mg/kg of body weight was given on day 9. The ethanolic extract of mint was continued to be given to the mice of that group from day 10 to day 16 of the experiment. Blood samples of the mice were obtained on the 17th day of the experiment by intracardiac sampling technique. Data was analysed by SPSS version 20.

RESULTS

When results were compared by one way ANOVA test among groups A, B and C, it was seen that highly significant differences in the serum values of malondialdehyde and glutathione peroxidase existed among the three groups (Table1).

Table 1. Comparison of serum malondialdehyde and glutathione peroxidase, among groups A, B and C, by one way ANOVA.

Parameters	Group A (n= 30)	Group B (n= 30)	Group C (n=30)	p-value
Serum malondialdehyde (ng/ml)	0.12±.037	0.22±.12	0.18±.05	0.00
Serum glutathione peroxidase (mg/dl)	1.03±.013	0.79±.12	0.94±.17	0.00

Values are presented as mean ± SD

*p< 0.00 highly significant.

Highly significantly raised serum levels of malondialdehyde were observed in mice of group B, in which a single oral dose of chloroquine was given. While highly significant decline in the serum levels of glutathione peroxidase was observed in the same mice. (Table 2) A highly significant decrease in serum malondialdehyde was observed in mice of group C, in which ethanolic extract of mint was given. Highly significantly raised serum levels of glutathione peroxidase were observed in group C mice. (Table 2)

Table 2: Comparison of serum malondialdehyde and glutathione peroxidase by post hoc Tukey test among the groups A, B and C.

Group comparisons	Serum malondialdehyde (ng/ml)	Serum glutathione peroxidase (ng/dl)
Group B versus Group A	0.00	0.00
Group Versus Group B	0.00	0.00

Values are presented as mean ± SD

DISCUSSION

The comparison of the results of serum malondialdehyde and glutathione peroxidase between group A and group B revealed a highly significant increase in the level of serum malondialdehyde, while a highly significant decrease in the level of serum glutathione peroxidase in group B as compared to that in group A. The highly significant elevated levels of serum malondialdehyde are indicative of lipid peroxidation because malondialdehyde is a secondary metabolite which is produced during the lipid peroxidation process. This shows that oxidative stress had been induced by chloroquine.

The serum levels of glutathione peroxidase were declined as a result of their excessive utilisation in scavenging the free radicals, which were produced in the lipid peroxidation process. These findings are consistent with those other studies.[15] For induction of hepatotoxicity in female Wister rats, they used chloroquine. The chloroquine was given at the dose of 970 mg/kg of body weight orally. Hydroperoxides and thiobarbituric acid reactive substances were found to be raised significantly. Their significance showed that a lipid peroxidation process had occurred. The other finding of their study was the decline in serum glutathione peroxidase level, which occurred due to the excessive utilisation of glutathione peroxidase in scavenging free radicals. Hence, it was proved that chloroquine, at the dose of 970 mg/kg, had induced oxidative stress in male albino mice.

Previous research has indicated that the ethanolic extract of mint contained an excessive amount of different antioxidants such as phenolic acids and flavonoids. Hence, in the current study, ethanolic extract of mint was used to see the antioxidant effect of mint.

The comparison of serum malondialdehyde and serum glutathione peroxidase between group C and group B revealed a highly significant decrease in malondialdehyde and a highly significant increase of glutathione peroxidase in group C mice as compared to those in group B mice. Reduced serum levels of malondialdehyde, a secondary metabolite of the lipid peroxidation process, showed a decline in the lipid peroxidation process by the ethanolic extract of mint. On the other hand, the raised serum glutathione peroxidase level showed that the ethanolic extract of mint had antioxidant potential. Hence, these findings indicate that mint (mentha) possessed antioxidant effects. Research work done showed that mentha arvensis possessed an antioxidant role. Their study revealed that the phenolic acids, which were antioxidants in nature, had hydroxyl groups which were capable of scavenging free radicals.[16-18] The antioxidants also could convert Fe^{+3} into Fe^{+2}, hence inhibiting the non-enzymatic lipid peroxidation process.

Similar findings were obtained by Wani et al, (2018).[19] Their results also showed that the ethanolic extract of mentha arvensis possessed the highest concentration of flavonoids and phenolic acids. These antioxidants could lose electrons due to which they were able to stop free radical chain reactions. The phenolic acids inhibited the conversion of Fe^{+3} into Fe^{+2}. Hence, these ended the non-enzymatic lipid peroxidation process.

Polyphenols are antioxidants, which are found in mentha arvensis in abundance. Their antioxidant actions include iron chelation and inhibition of xanthine

oxidase and NADPH oxidase, enzymes which generate reactive oxygen species. These inhibit lipoxygenase and cyclooxygenase and increase the formation of antioxidant enzymes.

CONCLUSION

Chloroquine, given at a dose higher than the therapeutic dose, induce oxidative stress. Mint possesses antioxidant potential against chloroquine induced oxidative stress.

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SK: Data collection, analysis, writing

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Original Article

FREQUENCY OF THROMBOCYTOPENIA AMONG DIFFERENT AGE GROUPS OF PATIENTS WITH LIVER CIRRHOSIS.

Salman Khan[1] , Muhammad Ayub Khan[2] , Zia Ullah[3] , Akhtar Nawab[4] , Wasim Akram[5] Shehzad Elahi[6]

ABSTRACT

Background: Patients with cirrhosis frequently exhibit thrombocytopenia, which might prompt an unnecessary referral for a bone marrow biopsy. The frequency of thrombocytopenia in liver cirrhosis is largely unknown in the study area. The main aim of our study is to evaluate the prevalence of thrombocytopenia in such patients in the study area.

Material and Methods: A retrospective cross-sectional study was conducted on 268 confirmed liver cirrhosis patients for thrombocytopenia in Khyber Teaching Hospital, Peshawar. Both, male and female subjects with established liver cirrhosis were included in the study while Patients had concurrent illnesses which can induce thrombocytopenia like malaria and dengue fever, ITP. This study takes a total of six months duration. Data analysis was done using SPSS software Version 20.

Results: Results showed that 74.8% of the studied subjects were diagnosed with thrombocytopenia while 25.2% were normal. Thrombocytopenic patients were also categorized into mild, moderate, and severe. Among different age groups, severe thrombocytopenia was found to be highest in all age groups having 113 (42.2%) subjects. Consequently, Severe thrombocytopenia in the age group 51-60 years was diagnosed in 99 (36.9%) cirrhosis patients which is statistically significant with a p-value =0.032. In addition, 56 (20.9%) of the age group 31–40 years age were diagnosed with severe thrombocytopenia.

Conclusion: Our study concluded that thrombocytopenia showed to be one of the diagnostic parameters while to check for complications, related to liver cirrhosis.

Keywords: Thrombocytopenia, Liver cirrhosis, Chronic Hepatitis, platelets

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INTRODUCTION

Thrombocytopenia, which is a common manifestation of liver cirrhosis, can be defined as a platelet count of <150,000 cells/μL.[1] Thrombocytopenia is further categorized as low (100 x 10^9), or moderate (<100 x 10^9). The severity of the liver disease is the main contributing factor despite its complex nature. In addition, a low platelet count is frequently a diagnostic indicator of cirrhosis and the existence of esophageal varices.[2]

The complication can adversely affect the treatment plan for liver cirrhosis by limiting the drug administration and delaying

surgical procedures.[3] As a result of cirrhosis, platelet sequestration in the spleen, bone marrow suppression, interferon-based treatments, and decreased thrombopoietin activity are all risk factors for developing thrombocytopenia (TPO).[4] TPO is mostly created in the liver, and thrombocytopenia can happen when platelets trapped in the congested spleen degrade more and more.[5] Autoantibodies against platelet surface antigens can encourage platelet sequestration and death by cells of the reticuloendothelial system in patients with liver dysfunction associated with hepatitis C.[6]

Cirrhosis is a histopathological diagnosis, although in patients with chronic liver disease (CLD), the presence of several clinical characteristics can point to cirrhosis, and liver biopsy is frequently unnecessary and dangerous.[7] Major indicators of underlying cirrhosis in clinical practice include a history of predisposing factors, the presence of stigmata of CLD, a palpable left lobe or a small liver span, splenomegaly, signs of liver decompensation, findings on abdominal imaging studies, laboratory data, and upper endoscopic findings.[8,9]

According to one study, cirrhosis can be accurately detected in 82–88% of CLD patients by using just a few ultrasonographic symptoms. However, the structural restrictions of this technique reduce the ultrasound's diagnostic efficacy.[10] In a sample of previously non-responders with chronic hepatitis C, a panel of serum fibrosis markers and regular laboratory tests were found to be useful in assessing the likelihood of histological cirrhosis.[11] Noninvasive testing has recently been proven to be effective at detecting severe fibrosis and cirrhosis.[12]

A fast-developing field is the pathogenesis of thrombocytopenia in chronic liver disease.[13] Previously, it was believed that thrombocytopenia was only brought on by portal hypertension-induced congestive splenomegaly, which sequestered the spleen. But now, there are numerous other hypothesized processes relating to platelet synthesis and destruction in cirrhosis.[14]

To date, the prevalence of thrombocytopenia in liver cirrhosis of different age groups is unknown in the study region. Routinely, we encounter several patients having severe thrombocytopenia in liver cirrhosis referred to us by a hematologist. The main aim of our study is to evaluate such prevalence in the study area.

MATERIALS AND METHODS

A retrospective cross-sectional study was conducted on 268 subjects from the Department of Medicine, Khyber Teaching Hospital, Peshawar on patients with liver cirrhosis. A written informed consent of the study was taken from patients included in the study. The study was approved by the Institutional Review Board of Khyber Teaching Hospital, Peshawar. All the samples were collected between 21 March 2021 to 21 Sept 2021 (six-month duration). The patients that were included in the study were identified for liver cirrhosis using the electronic medical record system of the hospital.

Both, male and female subjects with established liver cirrhosis were included in the study. The age margin used in the study was between 18-70 years. Patients having concurrent illnesses can induce thrombocytopenia like malaria, dengue fever, and ITP.

Detailed history and clinical examination were performed; an ultrasound abdomen showing serrated liver margins, dilated portal vein, and coarse liver was taken as cirrhosis. The clinical diagnosis of cirrhosis was made using combinations of several relevant clinical symptoms, such as ascites, splenomegaly, endoscopic evidence of esophageal varices, and/or radiologic evidence of cirrhosis.

5ml of blood sample was taken from the patient for lab detection of thrombocytopenia. A platelet count was performed using a hematology analyzer (Sysmex XP-100). All the samples, having a platelet count lower or higher than the

normal range (i.e. from 150k to 450k per microliter of blood), were confirmed by manual microscopic method.

Data analysis was done using SPSS software for Windows (Version 20.0, SPSS Inc. Chicago). Descriptive statistics were used to calculate the mean, standard deviation, and percentages of data. Thrombocytopenia was stratified among age, gender, duration of disease, and platelet count to see effect modifications. Post-stratification chi square test was applied keeping the P value equal or less than 0.05.

RESULTS

During the six-month study period, data of 268 patients were collected of which 164 (61%) were male subjects and 104 (39%) were female subjects. The mean age of the patients was 45.9 years. The body mass index (BMI) of each patient was calculated with average results of 24.7 for all 268 subjects (Table 1).

Table 1: Baseline demographics of the patients included in the study.

Total	268
Age in years (mean ± SD)	45.9± 17.3
Gender	
➢ Male-N (%)	164 (61%)
➢ Female -N (%)	104 (39%)
Average BMI	24.7

All the study subjects were divided into five different age groups. Similarly, thrombocytopenic patients were also categorized into mild, moderate, and severe. Among different age groups, severe thrombocytopenia was found to be highest in all age groups having 113 (42.2%) subjects. Consequently, 65 individuals were found to be normal (no thrombocytopenia) in all age groups (Table 2).

Severe thrombocytopenia in the age group 51-60 years was diagnosed in 99 (36.9%) cirrhosis patients which is statistically significant with a p-value =0.032. In addition, 56 (20.9%) of the age group 31–40 years age were diagnosed with severe thrombocytopenia. Mild thrombocytopenia was diagnosed in 34 individuals of the age group 51 - 60 years while 11 in the age group 31 - 40 years.

Table 2: Frequency of Thrombocytopenia (mild, moderate, and severe) among different age groups**.**

Platelet count	**20-30 years**	**31-40 years**	**41-50 years**	**51-60 years**	**61-70 years**	**Total N (%)**
Normal (150,00-400,000 cells/µL)	13	19	4	18	11	65 (24.2%)
Mild (101,000-140,00 cells/µL)	4	11	3	34	0	52 (19.4%)
Moderate (51,000-100,00 cells/µL)	5	10	4	16	3	38 (14.2%)
Severe (21,000-51,000 cells/µL)	2	16	35	31	29	113 (42.2%)
Total N (%)	24 (9%)	56 (20.9%)	46 (17.2%)	99 (36.9%)	43 (16%)	268

DISCUSSION

The most frequent and the first aberrant hematologic indicator to show up in cirrhotic patients is thrombocytopenia, which is followed by leukopenia and anemia.[15] Therefore, thrombocytopenia should be given the utmost consideration when assessing individuals with chronic liver disease. However, mild thrombocytopenia is the most typical manifestation in individuals with cirrhosis-related hematological abnormalities. Severe thrombocytopenia (platelet count 50,000) is common in cirrhosis with age > 40 years. It is observed that having a platelet count of 88,000 or less is related to having gastroesophageal varices.[16] In our whole study group, the mean platelet count was 69.9 33 k/uL, while in research participants with cirrhosis, it was 70.1 28.4 k/uL.

Although leukemia is considered a leading cause of thrombocytopenia, liver cirrhosis is now considered to be the most common contributing factor in patients aged>50 years.[17] Our study demonstrates that 36.9% of patients aged 51-60 years have the highest cases of thrombocytopenia compared to the other age groups. This demonstrates the fact that liver disease is more likely to affect the production of platelets in old age as compared to recent liver cirrhosis.[18]

The study demonstrates that about 74.8% of the total patients develop thrombocytopenia during liver cirrhosis. In previous literature different factors contribute to thrombocytopenia in liver cirrhotic patients, some of them include decreased activity of the thrombopoietin and hematopoietic growth factor, antiviral therapy, chemotherapy, and chronic hepatitis C virus infection's suppression of the marrow interferon-based therapy, which can help with the thrombocytopenia developing in cirrhotic patients.[19] Platelet sequestration in the spleen and decreased thrombopoietin production in the liver are the two main factors causing thrombocytopenia in liver cirrhosis.[20]

Our study emphasizes the importance of cirrhosis clinical diagnosis. The diagnostic process outlined in the literature may be deceptive and underestimates cirrhosis as a significant contributor to thrombocytopenia. Like this, there are currently no suitable recommendations for primary care doctors to direct thrombocytopenic patients to suitable subspecialties.[21] The study thus emphasizes the need for training medical professionals in the noninvasive diagnosis of cirrhosis and the correlation of metabolic syndrome symptoms with chronic liver disease.[22] Without understanding the diagnostic relevance of thrombocytopenia in cirrhosis, healthcare professionals may be led astray, which could have an impact on patient care overall and put more strain on available resources. It is significant to note that mortality rates related to the liver and overall, among people with NASH are higher.[23]

CONCLUSION

In summary, this study signifies the high prevalence of thrombocytopenia (75%) among liver cirrhosis patients and highlights the varying severity of thrombocytopenia across different age groups. These findings emphasize the importance of age-specific monitoring and potential interventions for managing thrombocytopenia in cirrhosis patients.

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AUTHOR'S CONTRIBUTION

SK:Introduction/objectives formulation
MAK: Literature search
ZU: Discussion
AN: Result and Analysis
WA: Data Collection and analysis
SE: Data Collection and analysis

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Origional Article

KNOWLEDGE, ATTITUDE AND PRACTICES REGARDING BLOOD DONATION AMONG MEDICAL STUDENTS OF A PRIVATE MEDICAL COLLEGE, LAHORE

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ABSTRACT

Background: Blood donation helps save the lives of millions. Voluntary blood donation is necessary for a sustainable blood supply. To determine the knowledge, attitude and practices of 1st to 5th year medical students about blood donation and their association with the sociodemographic factors in a private medical college.

Materials and Methods: A cross-sectional study was conducted among medical students from the first to final year from April to September 2022 after approval from the Ethical Review Board. Data was collected on a pretested self-administered questionnaire from all the students who were present on the day of data collection after obtaining verbal consent from them. The data was analyzed by SPSS-22. Chi-square/Fisher's exact was applied to find out the association of sociodemographic factors with knowledge, attitude and practices of blood donation. A p-value of ≤0.05 was considered as significant

Results: Out of 631 students, 601 who filled out the questionnaire had 0.5% good, 14.6% satisfactory and 84.9% poor knowledge. A positive attitude was reported by 80% of students. However, only 28.1% had ever donated blood. There is a statistically significant association of overall knowledge (p-value=0.01) and overall attitude (0.013) with the year of class. The practice of donating blood has a significant relationship with age (p-value=0.043), gender (0.000) and year of study (p-value=0.009).

Conclusion: The overall knowledge of medical students about blood donation was poor. The majority of the students had a positive attitude about blood donation and only more than one quarter had donated blood. There is a significant association of the academic year with knowledge regarding blood donation.

Key Words: Blood donation, Medical students, Knowledge, Attitude, Practices

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INTRODUCTION

Blood donation is one of the most important components of health care. Blood donation helps save the lives of millions of patients who are in dire need of blood. That is why voluntary blood donation is essential for sustainable blood supply.[1] It should be the responsibility of every country to have a proper national health strategy and network for the provision of safe and sufficient blood.[2] Globally around 118.5 million blood donations are made, out of which 40% of blood is donated by developed countries where only 16% of the population lives. The percentage of people who donate

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whole blood serves as a gauge for the country's overall blood supply. Blood donors can be classified as voluntary, paid and family or substitution donors which are also known as replacement donors. Over fifty percent of the world's blood donation is still obtained from paid blood donors and relatives in 54 different countries.[2] Less than 10 blood donations per 1,000 people are made every year in 66 nations, which is the rate usually considered indispensable to meet a country's minimal blood needs.[3] Only 10% of the blood collected in Pakistan comes from willing donors, even though 70% of the population is under the age of 29, and 90% comes from replacement donations made by families.[4] The World Health Organization (WHO) launched World Blood Donor Day in 2004 to raise awareness of the continued need for blood donations to save lives. Blood donations are essential to global health systems but can only be provided by voluntary blood donors.[5]

One of the reasons for blood scarcity is to inability to arrange regular blood supply as a result of misconceptions, hazards that donors perceive and lack of enthusiasm among them.[6] College students in Pakistan are a key source of voluntary blood donation if they are motivated and willing to do so.[7]

The purpose of the current study was to determine the existing knowledge, attitude and practice of medical students about blood donation and its association with sociodemographic factors.

MATERIAL AND METHODS

A descriptive cross-sectional study was conducted among the medical students of a private medical college from the first to final year from April to September 2022. All the students were included except those who refused to fill out the form and were absent on the day of data collection. The total number of students in the college was 630. A non-probability purposive sampling technique was applied. Age, sex and academic year constituted the independent factors, whereas knowledge, attitude, and practice served as the dependent variables. The data was collected by the students of Batch A1 of the fourth year on a self-administered pre-structured questionnaire comprising four sections after approval by the Institutional Review Board and by obtaining verbal consent from the students. Scoring of questions regarding knowledge and attitude was done. If the answer was correct then one mark was given and if it was wrong, then zero mark was given. There were 19 questions related to knowledge and 08 questions to attitude. The scores were categorized as Good, Satisfactory and Poor depending on the calculated percentage >75%, 60-75% and <60% respectively. Data was analyzed after cleaning the data by SPSS-22. Descriptive statistics were determined as mean and standard deviation for the age of participants. Categorical data was presented in terms of frequency and percentage of participants. The Chi-square /Fisher exact test was applied to demonstrate the relation of age, sex and year of education with knowledge, attitude and practice about blood donation. A P-value of ≤ 0.05 was taken as significant for this study.

RESULTS

Out of 630 medical students, 601 filled the questionnaire from whom 290 (48.2%) students were of 17-21 years of age with a mean age of 21.56 ±1.99 years. Two hundred and five (34.1%) were males and 283 (39.5%) healthcare workers were the primary source of information for blood donation. Out of 601 respondents, 3(0.5%) had good, 88 (14.6%) satisfactory and 510 (84.9%) had poor overall knowledge regarding blood donation. Whereas overall attitude was good in 362 (60.2%), satisfactory in 148 (24.6%) and poor in 91(15.1%) students (Figure-1).

Regarding knowledge related to blood donation, 516 (85.9%) out of 601 students knew the types of blood groups, 525 (87.4%) answered correctly about the

universal donor and the correct minimum age for blood donation (18 years) was mentioned by 460 (76.5%). However, 521 (86.7%) and 514 (85.5%) respondents did not know about the screening tests for blood donation and duration of preservation of donated blood respectively (Table- 1).

The majority of the students (80%) had a positive attitude about blood donation and almost 469 (78%) were willing to donate blood. Out of 601 students, 376 (80.2%) showed their willingness to donate blood to anyone (Table 1).

Regarding the practice of blood donation only 169 (28.1%) had ever donated blood and the frequency of blood donation once a year was reported by 128 (75.8%) students (Table-2).

Out of 432 students who had never donated blood,178 (41.2%) reported that one of the main reasons for not donating blood was that no one had ever approached them for this purpose, 156 (36.1%) perceived that they were unfit for blood donation, and 63 (14.5%) had fear of needles (Table -3).

There was a statistically significant association of the year of study with overall knowledge (Fisher exact test=16.48 & p-value=0.01) and attitude (Chi-square test=19.34 & p-value=0.013) (Table-4).

Whereas a significant relationship regarding blood donation practice was observed with age (p-value=0.043), gender (p-value=0.000) and year of study (p-value=0.009) (Table-5).

Figure No.1: Overall knowledge and attitude of medical students regarding blood donation (N=601).

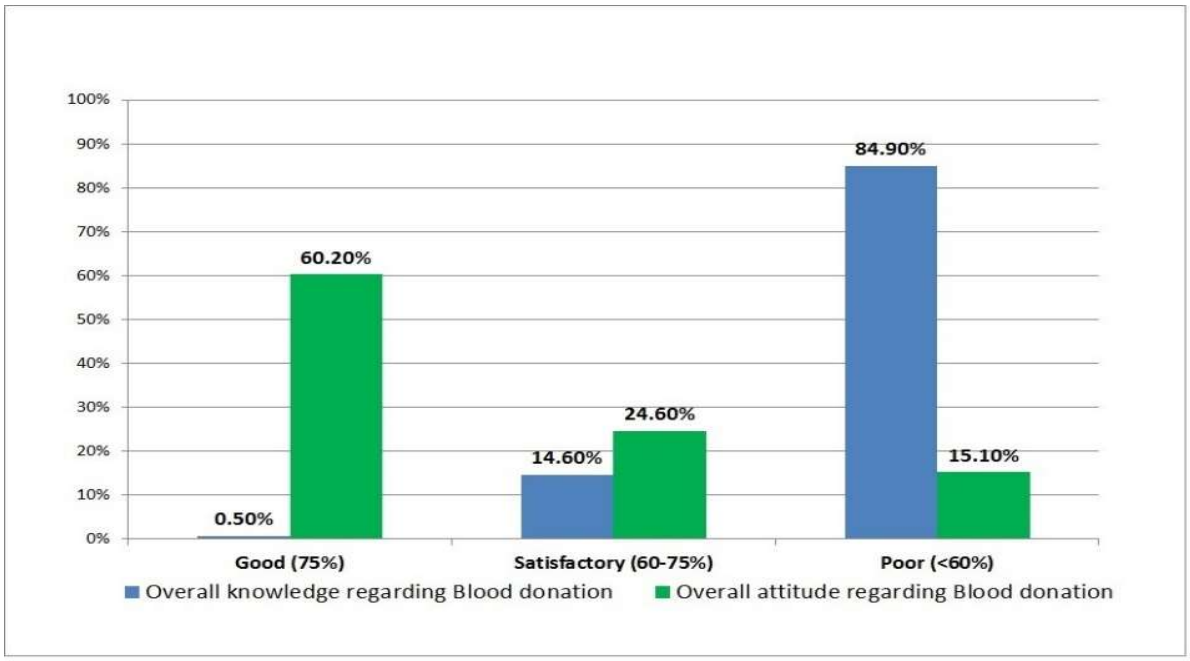

Table No. 1 Responses related to knowledge and attitutes about blood donation (N=601)

Knowledge questions	**Correct Answer**	**Incorrect answer**
1. Knowledge about different types of blood groups **(Yes)**	516 (85.9%)	85 (14.1%)
2. Universal recipient **(AB+ve)**	526 (87.5%)	75 (12.5%)
3. Universal donor **(O-ve)**	525 (87.4%)	76 (12.6%)
4. Minimum age for blood donation **(18 years)**	460 (76.5%)	141 (23.5%)
5. Maximum age for blood donation **(60years)**	155 (25.8%)	446 (74.8%)
6. Minimum weight for blood donation **(45kg)**	374 (62.2%)	227 (37.8%)
7. Hemoglobin for blood donation for females **(12.5mg/dl)**	469 (78%)	132 (22%)
8 Hemoglobin for blood donation for males **(13.5mg/dl).**	409 (68.1%)	192 (31.9%)
9. Blood donation by patients with chronic disease **(No)**	542 (90.2%)	59(9.8%)
10. Interval between blood donation **(3 months)**	391 (65.1%)	210 (34.9%)
11. Amount of blood donated at one time **(450 ml)**	248 (41.3%)	353 (58.7%)
12 No. of patients benefited by one unit of blood **(3)**	179 (29.8%)	422 (70.2%)
13. Transmission of infectious diseases due to unscreened blood **(All)**	17(2.8%)	584 (97.2%)
14. Screening tests for blood donation (**All)**	80 (13.3%)	521 (86.7%)
15. How many types of blood donors **(03)**	190 (31.6%)	411 (68.4%)
16. Duration of preservation of donated blood **(35 days)**	87 (14.5%)	514 (85.5%)
17. Donation time for one pint of blood **(6-8minutes)**	158 (26.3%)	443 (73.7%)
18. What are the types of blood donors (**Voluntary, paid & replacement)**	78 (13%)	523 (87%)
19. When can non-lactating women can donate blood **(after 6 months of delivery)**	314(52.2%)	7 (7.8%)
Questions related to attitude	**Correct answer**	**Incorrect answer**
1. People should donate blood **(Yes)**	569 (94.7%)	32 (5.3%)
2. Your attitude about blood donation (**Positive)**	481 (80%)	120 (20%)
3. Willing to donate blood **(Yes)**	469 (78%)	132 (22%)
4. If yes to whom like to donate blood **(Anyone)** (N=469)	376 (80.2%)	93(19.8%)

5. One can get infected while donating blood **(Yes)**	447 (74.4%)	154 (25.6%)
6. Best source of blood donors (**Voluntary)**	503 (83.7%)	98 (16.3%)
7. Any reward for blood donors **(No)**	454 (75.5%)	147 (24.5%)
8. Motivate other people for blood donation **(Yes)**	548 (91.2%)	(8.8%)

*Correct answers in bold letters

Table No.2: Practice of students about Blood donation (N=601)

Questions related to practices regarding blood donation	**Frequency**
1. Number of students who donated blood	
i) Yes	169 (28.1%)
ii) No	432 (71.9%)
2. Frequency of blood donation by students (N=169)	
i) Once a year	128 (75.8%)
ii) 2-3 times per year	41(24.2%)
3. When did the students last time donate blood (N=169)	
i) This year	94 (55.6%)
ii) Last year	54 (32%)
iii) More than one year	21 (12.4%)
4. To whom blood was donated: (N=169)	
i) Relatives	44 (26%)
ii) Friends	32 19%)
iii) Strangers (correct)	93 (55%)
5. Problems faced by students after blood donation: (N=169)	
i) Yes	26 (15.4%)
ii) No	143 (84.6%)
6. Feeling of satisfaction after blood donation: (N=169)	
i) Yes	141(83.5%)
ii) No	28 (16.5%)
7. Any reward for blood donors required (N=601)	
i) Yes	147 (24.5)
ii) No	454 (75.5%)

Table No. 3: Reasons recorded by students for not donating blood (432)

Reasons for not donating blood	**Frequency**
i) Never approached to donate blood	178 (41.2%)
ii)Unfit to donate blood	156 (36.1%)
iii) Fear of needles	63 (14.5%)
iv) Fear of knowing disease status on screening	29 (6.7%)
v) Risk of developing disease due to donation	44 (10.1%)
vi) Any other cause	14 (3.2%)

*Multiple responses allowed

Table No. 4: Association of knowledge and attitude with sociodemographic factors

Variables	**Knowledge**			**Attitude**		
	Good	**Average**	**Poor**	**Good**	**Average**	**Poor**
Age category						
17-24 years	2 (66.7%)	86(97.7%)	481(94.3%)	342 (60.1%)	138 (24.3%)	89 (15.5%)
25-33 years	1(33.3%)	2 (2.3%)	29 (5.7%)	20 (62.5%)	10 (31.3%)	2 (6.3%)
Fisher exact test:5.445 & p-value=0.064				**Fisher exact test:2.33 &p-value=0.31**		
Gender						
Male	1 (33.3%)	27 (30.7%	177(34.7%)	117 (57.1%)	49 (23.9%)	39 (19%)
Female	2 (66.7%)	61 (69.3%)	333 (65.3%)	245(61.9%)	99 (25%)	52 (13.1%)
Fisher exact test=0.688 & p-value=0.764				**Chi-square value:3.68 & p-value=0.162**		
Year of Study						
First-year	1 (0.7%)	34 (23.6%)	109 (75.7%)	78 (54.2%)	44 (30.6%)	22 (15.3%)
Second year	0 (0%)	08 (8.4%)	87 (91.6%)	66 (69.5%)	13 (13.7%)	16 (16.8%)
Third year	1 (1%)	17 (16.8%)	83 (88.2%)	64 (63.4%)	20 (19.8%)	17 (16.8%)
Fourth-year	0 (0%)	13 (11.6%)	99 (88.4%)	77 (68.8%)	23 (20.5%)	12 (10.7%)
Final year	1 (0.7%)	16 (10.7%)	132(88.6%)	77 (51.7%)	48 (32.2%)	24 (16.1%)
Fisher Exact test=16.48 & p-value=0.01				**Chi-square=19.34 & p-value=0.013**		

Table No. 5: Association of sociodemographic factors with donation of blood by medical students

Variables	**Yes**	**No**
Age categories		
17-24 years	155 (91.7%)	414 (95.8%
25-33 years	14 (8.3%)	18 (4.2%)
Chi-square= 4.085 & p-value=0.043		
Gender	**Yes**	**No**
Male	97 (57.4)	108(25%)
Female	72 (42.6%	324 (75%)
Chi-square=56.72 & p-value=0.000		
Year of Study	**Yes**	**No**
First year	42 (24.9%)	102 (23.6%)
Second year	16 (9,5%)	79 (18.3%)
Third year	25 (14.8%)	76 (17.6%)
Fourth year	44 (26.0%)	68 (15.7%)
Final year	42 (24.9%	107 (24.8%)
Chi-square=13.53 & p-value=0.009		

DISCUSSION

As blood donation is a resource that may save lives in a variety of emergency scenarios and other circumstances connected to it, this study is being undertaken in a private medical college to evaluate knowledge, attitude and practice in this area. Building a pool of enthusiastic young people is therefore vital to encourage them to give their blood freely and without payment.

In the current study, 84.9% of students had poor, 14.6% satisfactory and only 0.5% good overall knowledge regarding blood donation which was very disappointing as being medical students, they should have much better knowledge. Whereas studies conducted in Karachi [7], Lahore [8], Saudi Arabia [9] and Tamil Nadu [10] reported that 92.2%, 33.1%, 60.2% and 53.3% of medical students had adequate knowledge about blood donation respectively which is contrary to our study. The variation in overall knowledge in different studies may be due to whether the topic of blood donation is part of the curriculum or not, the number of blood donation campaigns held, the level of awareness and different criteria used for assessing knowledge regarding blood donation in these institutions.

A recent study reported that 80% of students had a positive attitude about blood donation. Other studies however stated 42%, 62.6% [6], 79.2 % [11] and 94.1% [12] positive attitude. The diversity of variation may be due to differences in motivation, level of awareness through social media, misconceptions and also due to holding of blood donation campaigns in these countries. In the present study, 78% of students said they would be willing to give

blood, and of those, 80.2% stated they would be willing to give blood to anybody. This shows that altruism has a key role in blood donation as confirmed by research studies done in Saudi Arabia [9], Lahore[13] and India[6] in which 83.9%, 77.7% and 91% respectively had reported their willingness to donate blood.

Out of 601 students, 75.8% had donated blood once and 24.2% had donated blood more than once whereas other studies depicted that 55.6 % [6] and 58.4%[14] had donated blood once whereas 44.4% [6] and 41.6%[14] had donated blood more than once. This variation in different studies indicates that necessary steps must be taken to motivate and inspire the students by creating awareness among them and by holding regular blood donation campaigns to provide opportunities for non-remunerated blood donation.

In the current study, a significant relationship between the academic year with knowledge (p-value=0.01) and attitude (p-value=0.013) respectively was reported. A study of Azad Kashmir reported that female respondents had greater overall knowledge (p-value=0.019) which is contrary to our study. Whereas a study of Lahore depicted that gender and age had a significant relationship with knowledge and only gender had a significant relationship with attitude which is contrary to our result[15].

A statistically significant association of practice of donating blood with age (p-value=0.043), gender (p-value= 0.000), and year of study(p-value=0.009) was depicted in the current study.

These results are in concordance with a study where a significant difference was observed in the history of blood donations for age (p=0.087), gender (p=0.0001), and educational level (p=0.0001).[15] Variables that were significantly associated with blood donation were age above 30 (p<0.001) and male sex (p=0.001).[16] The current study showed that third-year MBBS students donated the most blood, whereas first-year MBBS students donated the least. Whereas a study in Nepal reported that final year students (35.71%) donated blood more as compared to first year (8.57%) students.[14] Whereas a study in India reported that 16%, 32.7% and 44% of students who had donated blood belonged to the first year, the second year, and final year respectively.[17]

Reasons given in the current study for not donating blood were that 36.85% had never approached for blood donation, 32% perceived that they were unfit for blood donation as their knowledge about the criteria for donating blood was poor along with myths and misconceptions related to blood donation, fear of needles 13%, fear of knowing disease status on screening (6%) and risk of developing disease due to donation (9%). Similar reasons were also observed in other studies. [11 ,18] Thus, these findings are in concordance with our study. Thus there are two main challenges for improving non-remunerated voluntary blood donation which can be achieved by encouraging the youth population for blood donation and converting them to regular donors. According to a Saudi Arabia study, 70% of respondents stated that their college had never offered lectures regarding blood donation and never organized blood donation campaigns.[9] Thus role of medical colleges is very important for improving awareness and motivating the students for blood donation by holding seminars, and lectures related to blood donation and arranging blood donation campaigns.

A study conducted in Turkey among third and final year medical students reported that students who had taken part in blood donation training had much greater rates of blood donation and encouraged others to donate blood than those students who had not attended these trainings.[19] An international program called Club 25 was started in response to a WHO demand. It's a fresh idea that emphasizes the importance of saving lives by donating blood. Through Club 25, young people are inspired to visit a blood center, learn about healthy lifestyles, and routinely donate blood for 20

blood donations by the time they are 25. In 1989, Zimbabwe founded its first club. Over 60 nations have either started Club 25 programs or are attempting to find other ways to increase the number of young blood donors.[20] Such club 25 should also be established in Pakistan for the promotion of voluntary blood donation by young people.
A randomized control trial was conducted in Denmark to assess the impact of text messaging blood donors to let them be informed that their donation had helped a patient. It was found that text messaging increased the subsequent donation by 3.6% (p-value=0.023) who received the message as compared to those who did not.[21] Text messaging after post-donation among first time donors was an efficient intervention to increase the retention rate for subsequent donations as reported by another randomized control study. [22]
In a study conducted in Karachi, 69.7% of medical students answered positively that Short Message Service (SMS) reminded and motivated them to blood donation voluntarily more often. Thus SMS messaging can encourage persons to blood donation voluntarily by removing their fears and by increasing their awareness.[23]
Despite of thorough study, there are a few limitations in this study. Since the study is conducted on medical students only, the knowledge about blood donations among a common population cannot be assessed. Moreover, this study includes medical students from only one medical college so results cannot be generalized.

CONCLUSION

The medical students had poor knowledge about blood donation. Most of the medical students had a positive attitude towards blood donation and showed willingness to donate blood. One quarter of the students had donated blood.

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AUTHOR CONTRIBUTIONS

SA: Acquisition and analysis of data, drafting the manuscript
SH: Conception of idea & design of study, critically revised the article with intellectual input
ZB: Data collection and data entry and analysis of data.
AM: Data collection, record keeping, statistical analysis and drafting of manuscript

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Review Article

HISTORICAL AND CURRENT PERSPECTIVES ON THE HUMAN CONSUMPTION OF NON-NUTRITIVE SWEETENERS (NNS)

Syed Imran Ali Shah[1], Haleema Nawaz[2], Namra Nadeem[3]

ABSTRACT

Scientific discovery, consumer preferences, and regulatory concerns mark the trajectory of human introduction to nonnutritive sweeteners (NNS). Since the discovery of Saccharin by chance in the late 19th century, sweeteners such as Aspartame, sucralose, and acesulfame potassium were developed in the 20th century with their unique taste profiles with minimal to no caloric impact. As the demand for sugar alternatives grew, driven by health concerns and dietary considerations, NNS entered various food and beverage products. More recently, Stevia, a natural NNS derived from the leaves of the *Stevia rebaudiana* plant, has further diversified the market. However, debate about their safety and long-term health effects continues to shape research and public discourse. Regulatory bodies worldwide are continually striving to improve guidelines to ensure the safety of these sweeteners. The present review provides historical background, current status, and future outlook on the human consumption of NNS and summarizes their evolution to identify potential research areas.

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INTRODUCTION

Human taste receptors are adept at detecting sweet flavors,a trait that has led to the long-standing and enduring use of sweet substances in our food. These ingredients, derived from sugar cane, include syrups, molasses, and common table sugar. Notably, this inclination towards sweetness is evident even in human infants.[1] Nevertheless, the overconsumption of sugar and its byproducts can have significant negative impacts on human health, as demonstrated by the heightened risk of conditions such as heart disease and Type 2 conditions such as heart disease and Type 2 diabetes mellitus.[2] Consequently, sugar substitutes emerged as a seemingly safer alternative. Sugar free foods are extensively utilized and have gained popularity due to their low-calorie content.[3] While the term 'nonnutritive sweeteners' (NNS) is often used interchangeably with 'artificial sweeteners,' in the present context, the term is used to denote synthetically produced and naturally occurring NNS (Figure 1).

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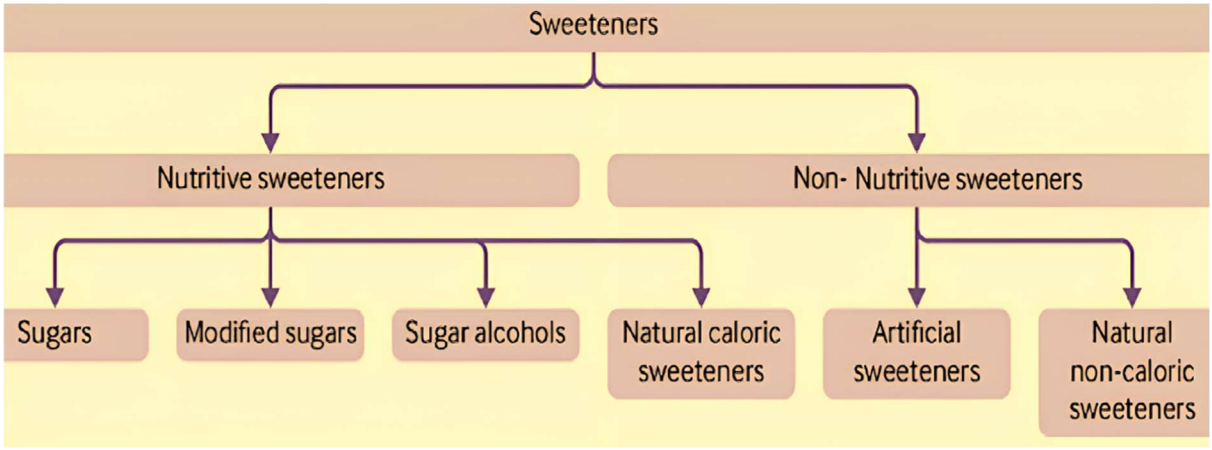

Figure 1. General classification of sweeteners

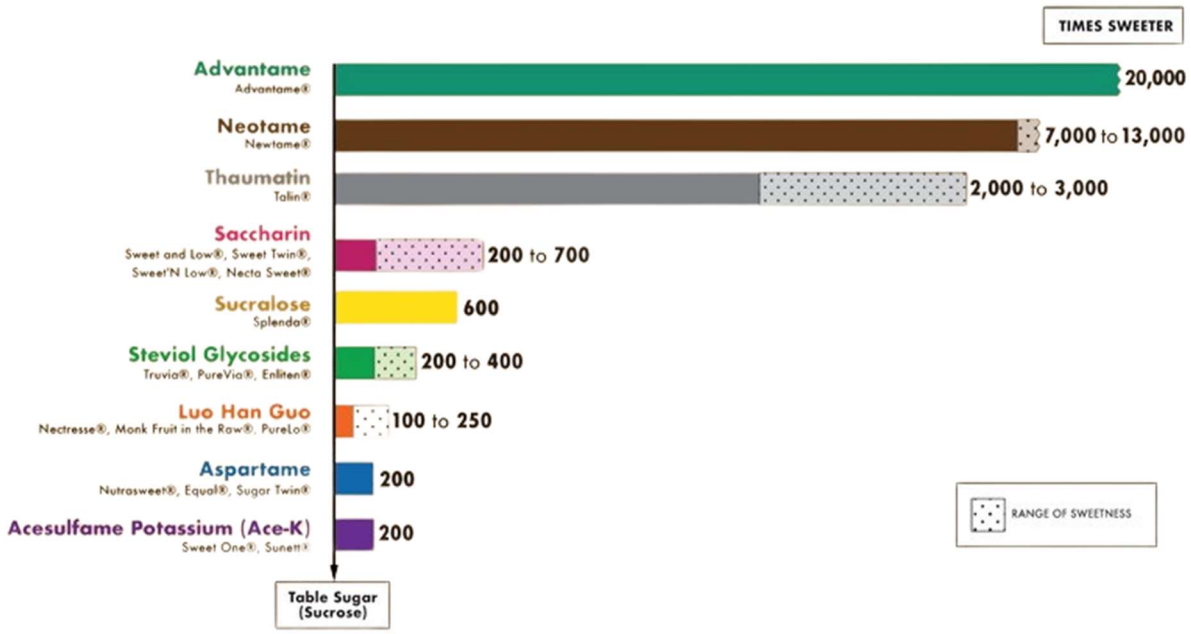

Figure 2. Comparison of sweetness intensities of various NNS and sucrose[4]

Substances with a calorific value of less than 2% that of sucrose per equivalent unit of sweetening capacity are termed NNS, whereas those with greater than 2% are termed nutritive. While the calorie content is negligible or zero, the sweetness intensity of different NNS is typically very high compared to sucrose (Figure 2)

For the present review, a comprehensive literature search was conducted using the keywords - "nonnutritive sweeteners" and "artificial sweeteners" to ensure a targeted exploration from the repositories of PubMed, Google Scholar, FDA (Food and Drug Administration), and dimensions from March 2023 to November 2023. Duplicate and redundant articles were excluded based on their titles. Data extraction following the keyword search was based on individual scrutiny of the retrieved articles from their title, abstracts, and full texts where necessary to identify those articles with historical background and consumption statistics of NNS. The review was synthesized based on the identified articles.

HISTORICAL STANDPOINT

NNS are food additives that provide the same sweet taste as natural sugar but significantly fewer calories, making them a zero or low-calorie alternative. NNS are present in various food items, including ice creams, beverages, chocolates, yogurt, jams, jellies, chewing gums, and salad

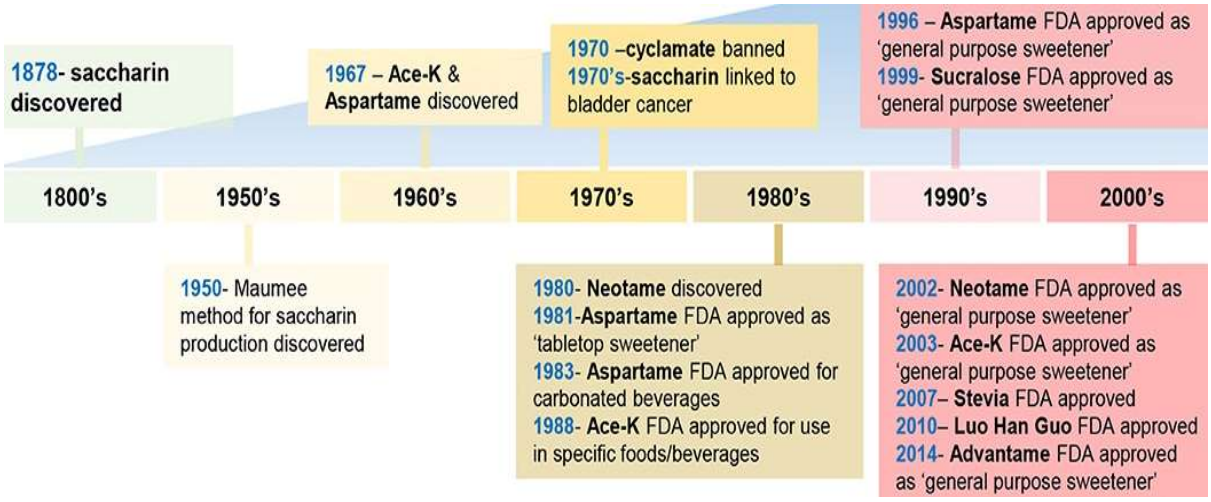

Figure 3. Discovery and approval timeline of NNS[5]

dressings. While NNS such as Alitame and cyclamate are utilized in some countries, the U.S Food and Drug Administration (FDA) has currently authorized the use of Saccharin, Sucralose, Aspartame, Acesulfame Potassium, Neotame, and Advantame. In contrast, the natural NNS, steviol glycosides, monk fruit (also known as Luo Han Guo), and thaumatin are 'generally recognized as safe' (GRAS).[4] The incorporation of NNS into the food industry began in the early 1800s, and that has continued to date with the addition of new entrants (Figure 3)

Saccharin was first discovered in 1879 during an experiment conducted by Constantine Fahlberg. Fahlberg was studying the oxidation mechanisms of toluene-sulfonamide when a splash accidentally landed on his finger. The sweet flavor he tasted upon licking his finger unveiled its potential as an alternative to sugar. Saccharin received FDA approval in 1970 and was widely used in soft drinks, chewing gums, toothpaste, mouthwash, and cosmetics.[6] However, certain studies conducted on rat models pointed to the potential development of bladder cancer linked to Saccharin use, prompting a prohibition on its use in Canada and a proposed ban in the USA.[7,8] Consequently, products containing Saccharin were mandated to carry a warning label in the USA for a period. Subsequent research into Saccharin's safety in humans led to the reversal of this decision by the USA in 2000. Health Canada also rescinded its ban on Saccharin in 2014, permitting its use in specific products.[9]

Aspartame was discovered accidentally in 1965 by James M. Schlatter during his research on anti-ulcer drugs. The FDA approved it as a table sweetener in 1981, and it received general-purpose sweetener approval in 1996. Aspartame has been the subject of extensive research as an additive in human food, and the FDA persistently reviews new scientific literature for updated information on Aspartame.[10] Acesulfame-K (acesulfame-potassium) was first discovered in 1967 by chemist Karl Clauss. It received FDA approval as a beverage sweetener in 1998 and later received generic approval in 2003. It is currently the most highly consumed NNS in diet soft drinks, dairy products, frozen desserts, and candy.[11] The British sugar company discovered Sucralose 'Tate & Lyle' in 1976 while searching for a chemical intermediate of sugar to use as a sweetener. The FDA granted it limited use approval in 1998, which was upgraded to general use in 1999.[12] Neotame, a derivative of Aspartame, was approved in 2002 as a general-purpose sweetener. Because of its heat-stable properties, neotame is an effective alternative to sugar for baking purposes.[13] Advantame is a relatively new

entrant to the list of NNS and was approved by the FDA for use as a general-purpose sweetener in 2014. Like Neotame, Advantame is also heat-stable and is used in sugar-free baked products.[14]

Steviol glycosides, found in the leaves of the Stevia rebaudiana (Bertoni) plant, native to certain regions of South America and commonly referred to as Stevia, are natural sweeteners. High-purity stevia derived sweeteners such as Rebaudioside A, Stevioside, and Rebaudioside D are considered safe by the FDA.[15] However, using stevia leaf and unrefined extracts is not deemed GRAS, and their distribution in the U.S. as sweeteners is prohibited.[16]

Thaumatin is a collection of highly sweet basic proteins extracted from the Thermococcus Danielle fruit, also known as the West African Katemfe fruit. Thaumatin has food additive authorization in the European Union (E.U.). It is used as a sweetening agent in various such as jams, ice cream, baked goods, potato-based snacks, and breakfast cereals; the plant known as Siraitia grosvenorii (Swingle), more commonly referred to as Luo Han Guo or monk fruit originates from Southern China. The China Food and Drug Administration (CFDA) approved its use as a food sweetener in the 1990s. Extracts derived from this fruit are typically used as sweeteners for general purposes and also as tabletop sweeteners.[17]

GLOBAL CONSUMPTION OF NNS

In recent years, there has been a significant surge in the consumption of NNS. An examination of Euromonitor sales data over 12 years from 2007 to 2019 revealed a 36% rise in the per capita quantities of NNS sold through beverages globally.[18] In the USA, data from the National Health and Nutrition Examination Survey (NHANES), collected from 1999 to 2008, reveals that consuming beverages containing NNS rose from 18.7% to 24.1% among adults and from 6.1% to 12.5% among children.[19] A similar study using NHANES data from 2003 to 2010 also found an increase in NNS consumption, with adults consuming NNS containing beverages rising from 21.1% to 24.9% and children increasing from 7.8% to 18.9%. In the same study, data collected from Neilsen Homescan (Neilsen Co.) between 2000 and 2010 revealed a rise in purchases for products containing low-caloric sweeteners, particularly in households with children.[20] As of the year 2019, beverages continue to be a concerning source of NNS intake for children in the U.S., with over 70% of drinks advertised to children containing NNS.[21] While NNS are used globally, dependable estimates of the prevalence of NNS consumption at the national level are primarily restricted to data from the United States. In 2023, the worldwide sweeteners market was valued at USD 102.2 billion. It is projected to reach approximately USD 144.86 billion in a decade (Figure 4), with a compound annual growth rate (CAGR) of 3.6% from 2023 to 2032.[22]

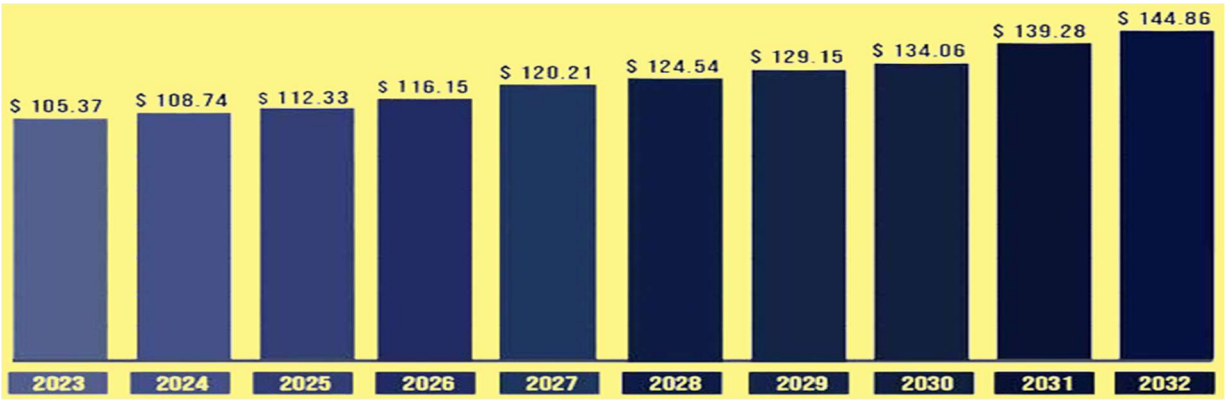

Figure 4. The projected global market value of nonnutritive sweeteners[22]

HEALTH IMPACT AND FUTURE OUTLOOK

NNS has been shown to exhibit clinical benefits, particularly in weight management and glycemic control. By imparting sweetness without affecting the caloric content of food, NNS is a productive tool for individuals requiring weight management. This attribute is particularly relevant for populations contending with obesity and diabetes. Furthermore, NNS offers individuals with specific dietary constraints, such as those who have diabetes, the opportunity to indulge in sweetened products without potentially compromising glycemic equilibrium.[23] However, the current clinical utilization of NNS is not devoid of complexities, and research on NNS continues to be dynamic, revolving around the long-term health effects of NNS consumption.[24] Despite being considered safe by regulatory authorities, there is an ongoing interest in understanding their impact on metabolic health, gut microbiota, and potential links to chronic conditions.[25] The development of novel NNS formulations, seeking alternatives that mimic sugar's taste and sensory experience without compromising health, is another area under exploration. The environmental implications of widespread NNS use, such as their production processes and disposal, are also worth assessing in the context of sustainability. Likewise, behavioral studies examining consumer perceptions, preferences and adoption of NNS is another avenue for research.[26-28]

CONCLUSION

In conclusion, the historical development and contemporary consumption patterns of NNS from the accidental discovery of Saccharin in the late 19th century to the modern diversification with alternatives like Aspartame, sucralose, acesulfame potassium, and Stevia, these sweeteners have become ubiquitous in the realm of sugar substitutes. The ever-growing demand for healthier alternatives has driven their integration into many food and beverage products. However, as consumption patterns rise, so do concerns about safety and long-term impacts. In this context, the regulatory landscape is pivotal in establishing guidelines on using NNS to safeguard public health. A multifaceted approach is required to comprehensively understand NNS, addressing both the scientific and societal dimensions of their use.

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AUTHOR CONTRIBUTION

SIAS: Conceived the review, reviewed & finalized the manuscript
HN: Conducted the literature search, revised the draft, reviewed & finalized the manuscript

NN: Conducted the literature search, wrote the first draft of the manuscript, reviewed & finalized the manuscript.

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Case Report

PHEOCHROMOCYTOMA WITH NEUROFIBROMATOSIS TYPE 1

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ABSTRACT:
Patients with early onset hypertension should be evaluated for secondary causes so that the underlying pathology can be ruled out effectively, we present a case of 25 years old female having pheochromocytoma with neurofibromatosis type 1. The patient presented in OPD with a complaint of episodic hypertension with palpitations and tremors for 7 months, the patient had this problem for almost 6 to 7 years. The patient was admitted to the hospital, and examination and initial workup were done leading to a diagnosis of pheochromocytoma. The patient's episodic complaint was treated with anti- hypertensive medications.

Keywords: pheochromocytoma, neurofibromatosis, antihypertensive medication.

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INTRODUCTION

Pheochromocytomas are rare Neuro-endocrine tumors occurring in about 2 to 8 out of every one million people that produce catecholamines. Approximately 80% of these tumors occur in the adrenal medulla (phaeochromocytomas), while 20% arise elsewhere in the body in the sympathetic ganglia (paragangliomas).[1] Mostly present as benign lesions others approximately 15% show malignant features. Around 40% are associated with inherited disorders, including neurofibromatosis, von Hippel–Lindau syndrome and sometimes as a part of MEN 2.1 Only 27 cases have been reported in Pakistan in the past two decades. Significantly 7 cases in 2021 and 4 in 2022. These tumors usually cause paroxysmal significantly 7 cases in 2021 and 4 in 2022. These tumors usually cause paroxysmal hypertension sometimes acute, and can also lead to serious health problems including stroke, and myocardial infarction.[2]

My patient started having paroxysmal episodes of headache, Palpitations, facial pallor and sweating off and on 5 years back. These were associated with severe abdominal pain, nausea and sometimes vomiting. She was found to have very high readings during these episodes. The episodes were relieved by oral antihypertensive and sometimes required IV antihypertensive treatment by local paramedics. She remained asymptomatic for a couple of years, only 2 episodes were experienced by her in the past 2 years after which she started having more frequent and severe episodes since the start of 2023. She also complains of a large raised firm tender nodule on the posterior aspect of the middle third of her left arm, measuring approximately 15cm x 12cm. It was initially small and gradually increased in size over the past 10 years.

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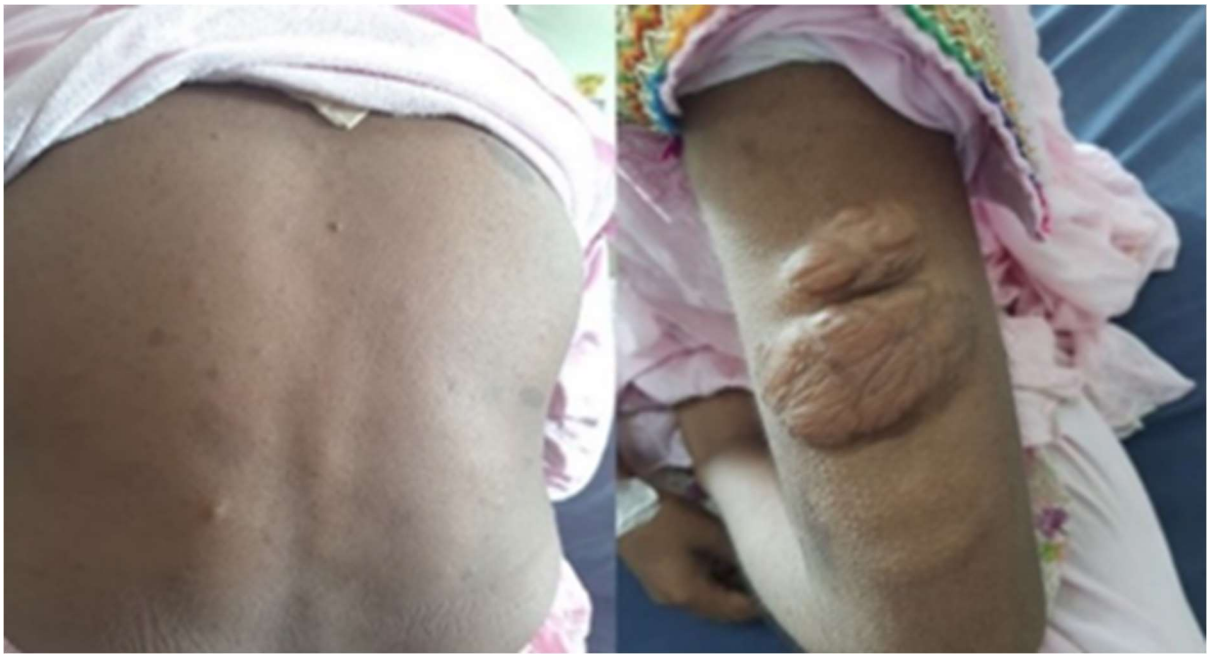

Fig 1: showing café au lait macules and small neurofibromas present on the back of the patient. A large neurofibroma is present in the middle of the left arm.

Small nodular lesions were also noticed on her face, arm and back, and hyper pigmented macules (café au lait) were present in the upper and middle back as well as her left arm as shown above. Freckles were present in axillary areas bilaterally. Her mother had the same nodular lesions on her body, she died a few months back due to some unexplained cause at the age of 55 years according to the patient. Her brother also has the same nodular lesions and hyper pigmented macules on his entire body as shown below. But he never**.**

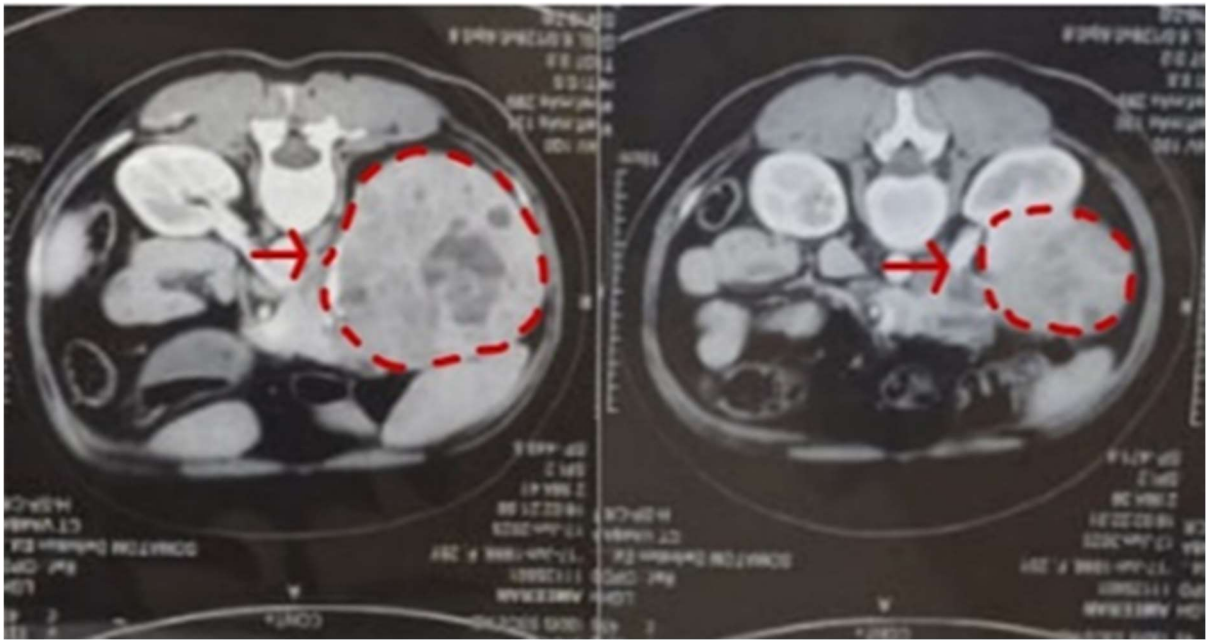

Fig 2: showing neurofibromas on different body parts of the brother of the patient.

Experienced symptoms like palpitations, and episodic hypertension, signifying tumors can be asymptomatic.[3] On abdominal ultrasound a large complex retroperitoneal mass was seen in the right lumbar region with echogenic foci and thin septations, Doppler showed minimal vascularity. It appeared to be originating in the anterior pole of the right kidney, a differential of pheochromocytoma was suggested by the radiologist. On computed tomography scan of the abdomen and pelvis(shown below), there was a 10x11x11 cm well-defined solid lesion in the right supra renal retroperitoneal location, the contralateral adrenal gland appeared to be unremarkable it was demonstrating internal foci of necrosis, compressing upon the right lobe of the liver. The lesion was also compressing upon and displacing the right kidney in the caudal direction, mass effect was also noted on IVC, The Liver was unremarkable for any focal lesions abdominopelvic vasculature was patent. There was no significant lymphadenopathy. The visualized skeleton was unremarkable for any osseous lesions.

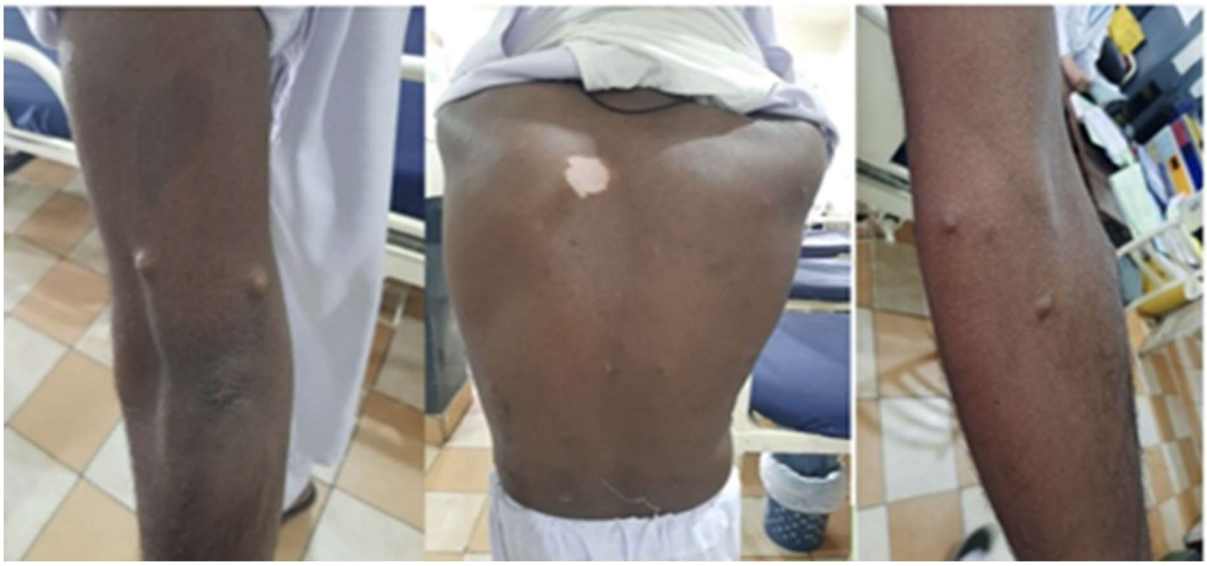

Fig 3: CT scan of the abdomen showing mas in retroperitonal location. Compressing surrounding visceras.

24 hours urinary metanephrine levels were raised significantly to 1859.78 micrograms/day (N=350 microgram/ day). The patient required stat doses of inj. labetalol 10mg to control episodes of hypertensive urgency. She was managed by controlling the Blood pressure with alpha and beta blockers. She was initially started on the tab. Doxazosin 4mg 1 x PO x OD. After 2 days tab. Carvedilol 6.25mg 1x Po x BD was added to her treatment regimen. Her Blood pressure was monitored 6 hours daily. On surgical evaluation by a consultant surgical Oncologist Laparoscopic Adrenalectomy was advised but unfortunately despite extensive counselling the patient was reluctant to get an invasive procedure and opted to be managed medically for symptomatic relief of her paroxysmal episodes of headache and palpitations only, hence she was discharged on alpha blockers and beta blockers therapy and was advised weekly follow up. The patient was followed every week henceforth in opd for 2 months where she reported no further symptoms since discharge indicating that her symptoms were adequately controlled with medical intervention, the patient remains in close follow up regarding her condition.

DISCUSSION

This case presents a unique clinical scenario of a young female patient with a rare combination of pheochromocytoma and neurofibromatosis type 1.[4] The coexistence of these two conditions poses diagnostic challenges and underscores the importance of considering secondary causes in cases of early-onset hypertension. The patient's initial presentation of episodic hypertension, palpitations, and tremors, lasting for 7 months and recurring over the past 6 to 7 years, prompted further investigation.[5] The identification of a large retroperitoneal mass on imaging, coupled with significantly elevated urinary metanephrine levels, led to the diagnosis of pheochromocytoma. This neuroendocrine tumor, originating from the adrenal medulla, is known for its potential to produce catecholamines, resulting in episodic symptoms of hypertension. Of particular interest in this case is the concomitant presence of neurofibromatosis type 1, a genetic disorder characterized by nodular lesions, hyper pigmented macules, and café au lait spots. The patient's family history revealed a similar condition in her mother and brother, adding a familial aspect to the case.[6] The mother's unexplained death at the age of 55 raises questions about the potential impact of these conditions on overall health and lifespan. The decision-making process regarding the management of this patient's pheochromocytoma is noteworthy. While laparoscopic adrenalectomy was recommended, the patient opted for medical management due to concerns about undergoing an invasive procedure. This decision emphasizes the importance of patient preferences and the need for individualized approaches in the management of complex cases. The successful control of the patient's symptoms with alpha and beta blockers highlights the efficacy of medical intervention in certain cases of pheochromocytoma. The rarity of this case, especially in the context of neurofibromatosis type 1, contributes to the existing body of knowledge on the manifestations of genetic disorders and their intersection with rare endocrine tumors. In conclusion, the interplay between pheochromocytoma and neurofibromatosis type 1, along with the challenges in decision-making and management, adds a layer of complexity to this clinical scenario, emphasizing the need for a multidisciplinary and patient-centred approach.

CONCLUSION

This case highlights the rare co-occurrence of pheochromocytoma and neurofibromatosis type 1, presenting with episodic hypertension. The patient's familial history adds complexity, emphasizing the importance of genetic factors. Medical management effectively controlled symptoms, showcasing the role of tailored interventions. The patient's preference for non invasive treatment underscores the need for individualized care. This case contributes valuable insights into the intricate interplay of genetic disorders and rare endocr intumors, emphasizing the significance of a multidisciplinary approach.

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CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

AUTHOR'S CONTRIBUTION

ZI: Introduction & review
SI: Abstract, manuscript writing
HA: Case history, bibliography & review
AYY: Discussion writing & conclusion

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