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Editorial

Clinical Supervision: A Way Forward to Residents' Professional Development

Uzma Ahsan

Clinical supervision is defined as "the formal provision, by senior qualified health practitioners, of an intensive one to one relationship-based education and training that is case focused and which supports, directs and guides the work of trainees".[1] It has been the least researched and supported aspect of medical education and yet is fundamental to the effective training of physicians.[2] Global research identifies the integral value of clinical supervision and agrees to the fact that it is of utmost importance to ensure a safe clinical specialist to the society.[3]

The process of clinical supervision plays a critical role in building and carving future consultants, specialists, and clinical supervisors through postgraduate medical training programs. Kilminister et al. complement the definition by mentioning that "supervision includes provision of ongoing feedback on matters of personal, professional and educational development in the context of patient doctor care".[3]

In health professionals, supervision integrates four major domains, i.e., instructive, supportive, managerial, and supervising patient management and care. A clinical supervisor is a designated senior professional or a mentor who aids, directs, monitors, supervises, appraises, and assesses a junior colleague's learning and development, research work, academic performance, personal, interpersonal, and professional progress with structured frequent interactions and communications.[4] The trainee is the medical graduate or junior doctor who is enrolled in a specialty training program approved by the degree awarding bodies and is fulfilling the requirements of clinical training.[5] The supervisor and the trainee are connected with an emotional link and a hierarchal working relationship that transforms and progresses through time and could be negatively impacted by a mismatch between personalities and attitudes. The pivotal position of a favorable supervisor-trainee relationship in preventing medical errors and ensuring patient safety is indisputable. However, the power structure of the supervisor-trainee alliance shifts a greater burden of responsibility towards the supervisor. Good supervisor-trainee alliances are featured by general compatibility on goals and objectives, professional perspectives, ethical values, mutual respect, cultural backgrounds, and abilities to marginalize the differences.[6]

Clinical supervision could be of three kinds; effective or adequate, ineffective, inadequate or bad, and harmful supervision. The skills, approaches, and behaviors of the supervisors are the differentiating components of various types of clinical supervision received by a trainee.[7] Effective supervisors are knowledgeable, skillful, and competent professionals who can successfully demonstrate their expertise in guiding the trainee, ensure successful transfer of clinical skills and techniques, and are aware of the limitations of their expertise and knowledge. Honesty, confidentiality, impartiality, tolerance, and patience are intrinsic behaviors of successful and effective supervisors. Trainees expect professional trust, respect, support, encouragement, and empathy from good clinical supervisors. Trainees correlate good supervision with the ability of the supervisor to allocate time for direct interaction, sense of responsibility and approachability, and be concerned for personal issues of the trainee and permissiveness for feedback about supervision. Competent supervisors can manage and organize the trainings to invigorate the problem solving, decision making, and teamwork skills in the trainee; arouse intellect, out of the box thinking, gradual autonomy, and steering them step by step to complete independence.[8] Ideal supervisors use supervisory contract, set up training goals and objectives, clarify professional limitations to the trainee, continually monitor a trainee's performance, personally provide consistent positive and negative feedback in a clear format. On the other hand, a good trainee must have a positive attitude, the strength to accept challenges, and the capability to solve problems. A trainee should be respectful, non-antagonistic, adaptable, appreciative, uncritical, non-manipulative, and able to work in teams.[9]

Ineffective or inadequate supervision is characterized by lack of suitable clinical skills, knowledge, or approach for teaching, guidance, and mentoring;

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ineffective demonstration and transference of clinical skills; and non-systematic, non-specific, and inconsistent feedback. Inadequate supervisors are often extremely busy, inaccessible, unreliable, assertive, non-supportive, intimidating, discriminatory, and unable to create and sustain positive learning environment.[10] Lack of motivation, commitment, and accountability in clinical supervisors often leads to ineffective or inadequate supervision. However, it is important to realize that ineffective, inadequate, or bad and harmful supervision could also be due to negative attitude of the trainee. The trainee might not have the capabilities or motivation, or suffers from a lack of the fundamental skillfulness, suitable characteristics, and aptitudes, or has a personality clash with the supervisor.[11]

The aim of producing good clinical professionals with a desirable blend of skills, knowledge, and behaviors, necessitates positive role modelling by the supervisors, because positive role modelling has a pivotal place in clinical training. Clinical trainees learn by both instructional and reflective apprenticeship. Therefore, clinical trainees acquire knowledge, skills, and practice by cognitive as well as non-cognitive manners.[2]

A positive role model supervisor can develop and construct good professionals, scholars, medical educators, with ingrained attributes of critical thinking, objective analysis, self-evaluation, and self-responsibility. In the local context, there is a need for improvement in terms of standardized training of residents enrolled in various postgraduate programs.[11] Role of the degree awarding authorities for regulation of the supervisory relationship and practices cannot be underestimated. Ineffective supervision or lack of interest in supervision may be linked to supervisor's busy schedules, burden of work, and inherent attitudes. A better regulatory framework with administrative reforms and a balanced workload between a supervisor's administrative and academic responsibilities is required to enhance the quality of clinical supervision.

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Original Article

Bone Tumors & Expression of SATB2 Immunohistochemical Stain in the Diagnosis of Osteogenic Sarcoma

Madiha Arshad, Shahida Niazi, Safeena Sarfraz

ABSTRACT

Objective: To determine the frequency & histopathological features of various benign and malignant bone tumors at Mayo Hospital/KEMU, Lahore. To evaluate the expression of Special AT-rich sequence-binding protein 2 (SATB2), a new novel immunohistochemical stain in the diagnosis of osteogenic sarcoma.

Methodology: It was a retrospective cross-sectional study of 83 cases of bone tumors received and reported from 1st July 2017 to 30th June 2019 at Mayo Hospital/ KEMU, Lahore. Relevant records & data, radiological findings, paraffin wax blocks & slides were retrieved and reviewed. Fresh slides were prepared wherever required. The immunohistochemical (IHC) stain of SATB2 was applied to the relevant malignant cases. Data was compiled & analyzed using Statistical Package for the Social Sciences (SPSS) version 23.0.

Results: Out of a total of 83 bone tumors, 56(67.46%) cases were classified as benign & 27(32.53%) cases as malignant. Four cases in the malignant category were metastatic in origin. The commonest benign tumor was osteochondroma comprising of 24(42.85%) cases followed by giant cell tumor consisting of 16(28.57%) cases. The most frequent malignant bone tumor was osteosarcoma composed of 14(51.85%) cases & chondrosarcoma was the 2nd commonest malignant tumor comprising of 5(18.85%) cases. In the benign cases, the age of the patients ranged from 12 to 47 years & in the malignant cases, the age ranged from 5 to 67 years. Nine (64.29%) cases of osteosarcoma were reported in children in the age range of 5 to 16 years. Overall humerus was the bone, most frequently affected by both benign & malignant bone tumors comprising 24(28.90%) cases followed by the femur which was affected in 21(25.3%) cases. Nuclear positivity for SATB2 was noted in 12(87.5%) cases of osteosarcoma.

Conclusion: Osteochondroma is the commonest benign bone tumor and osteosarcoma is the most frequent malignant bone tumor. The immunohistochemical stain SATB2 proves to be a very effective tool in the diagnosis of osteosarcoma.

Keywords: *Bone tumors. Immunohistochemical stain SATB2. Osteosarcoma. Osteochondroma.*

INTRODUCTION

Bone is a rigid firm mesenchymal body tissue comprising of cells embedded in a mineralized organic matrix having the tendency to develop a neoplasm either benign or malignant.[1] Bone tumors comprise only 0.5% of the total world cancer incidence.[2] When excluding myeloma and lymphoma bone tumors with a malignant biological tendency, constitute only 0.2% of all malignancies in adults and 5% of childhood malignancies.[3,4] They may be classified as 'primary tumors', which arise in the bone itself, and 'secondary tumors' which originate elsewhere from some other site and then produce their metastatic deposits to the skeleton.3 Thus bone tumors frequently cause a diagnostic challenge for general surgical pathologists. Clinical findings and radiological data are of utmost importance in accurately diagnosing malignant bone tumors for determining stage, prognosis & planning limb salvage surgery.[5] These tumors tend to occur at certain favored skeletal sites and in particular age groups or even specific locations in particular bones.[6] Secondary or metastatic bone tumors however involve the axial skeleton more frequently than the appendicular skeleton. Relevant data on age, gender, precise anatomical location of the lesion, and radiological correlation are of utmost importance in arriving at a definitive histological diagnosis.[7]

Advancements in the field of immunohistochemical stains and molecular studies have certainly made an accurate histological diagnosis of bone tumors much easier. Special AT-rich sequence binding protein (SATB2) is a potent nuclear matrix protein associated transcription factor and epigenetic regulator enhancing osteoblastic differentiation and bone regeneration.[8] It is also expressed in the glandular epithelial cells of the lower gastrointestinal tract thus facilitating the diagnosis of colorectal cancer too.[9] Hence SATB2 has now emerged as a new novel immunohistochemical stain for the confirmation of osteogenic sarcoma and to differentiate it from its potential mimickers.[8]

Diagnosis of osteosarcoma is a challenging task due to limited osteoid deposition in some cases and the presence of hyalinized stroma which mimics osteoid. In these cases, SATB2 plays a significant role in its diagnosis. The present study was conducted to determine the frequency of bone tumors in our setup & to evaluate the diagnostic utility of SATB2 in osteogenic sarcoma.

METHODOLOGY

The present study was a two year retrospective analysis on all bone tumors received at the Department of Pathology, King Edward Medical University, Lahore, Pakistan from 1st July 2017 till 30th June 2019. The study

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was approved by the ethical committee of the institution. All the histopathological reports of these cases, maintained in the histopathological section, were reviewed and Haematoxylin and Eosin (H & E) stained slides of every case were re-examined. Further sections were cut from paraffin blocks wherever required. The slides were re-examined to analyse data regarding age, gender, anatomical location, radiological findings, and histopathological diagnosis. All cases irrespective of their age and gender were considered. Hematological malignancies like myelomas and lymphomas were excluded from the study as their diagnosis requires bone marrow biopsy. Inflammatory bone lesions, tumor-like conditions, and primary tumors of odontogenic origin were also excluded from the study.

In suspected and diagnostically uncertain cases of osteogenic sarcoma & its variants showing scanty osteoid, the immunohistochemical stain SATB2 was performed according to the instructions and guidelines of the kit (Dako Company). The stained slides were examined under the microscope to confirm the diagnosis and to exclude other bone sarcomas that cause diagnostic confusion like lymphomas, Ewing sarcoma family of tumors (ESFT), chondrosarcoma, and aneurysmal bone cyst.

STATISTICAL ANALYSIS

Data was analyzed using Statistical Package for the Social Sciences (SPSS) version 23.0. Frequency and percentages were calculated.

RESULTS

In the present study, a total of 104 bone lesions were reported over a period of two years. Out of 104 bone lesions, 83(79.8%) were bone tumors. Inflammatory bone lesions & tumor-like conditions constituted 21(20.2%) cases and were excluded from the study. Benign tumors accounted for 56 (67.46%) cases and malignant tumors for 27(32.53%) cases giving a benign to a malignant ratio of almost 2:1. Out of the 27 malignant bone tumors, 23(85.18%) cases were of primary bone origin and 4(14.81 %) cases were metastatic in origin (Table 1).

The mean age of the patients presented with bone tumors was 23±3.1 years and age ranged from 5 to 67 years. Sixty one (58.7%) patients were males and 43(41.3%) were females. In the benign category, patients ranged in age from 12 to 47 years whereas in the malignant group patients age ranged from 5 to 67 years.

Considering the benign category of bone tumors, the commonest tumor reported was osteochondroma which consisted of 24(42.85%) cases out of 56 benign bone tumors and its most common site was the upper end of the humerus (13 cases) followed by the lower end of the femur and tibia (5 cases each). Only one case of osteochondroma was reported in the scapula (Table 1 & 2). Cases of giant cell tumor constituted 16(28.57%) cases of which 7 were reported in the femur and 3 each in the humerus and tibia, 2 in the radius, and 1 in the ulna. The frequency and site of distribution of other benign tumors are shown in Table 1 and 2. Microscopic pictures of benign tumors are shown in Figure 1, 2 & 3.

Regarding the malignant bone tumors which comprised of 27 cases, the commonest primary malignant tumor was osteogenic sarcoma composed of 14(51.85%) cases. In 5 cases, the distal femur was involved, 3 cases were reported each in the tibia and humerus, 2 cases in the ulna, and 1 case was reported in the radius. Chondrosarcoma was the second commonest malignant bone tumor constituting 5(18.51%) cases. Two cases were reported in the distal femur and 1 each in the jaw, ulna, and rib (Table 1, 2). Two cases of Ewing sarcoma family of tumors were located in the shaft of the radius and 1 in the humerus. The single (3.70%) case of chordoma was located in the sacrum. The anatomical location of these cases is depicted in Table 2. Figure 4, 5, 6 & 7 showed microscopic picture of

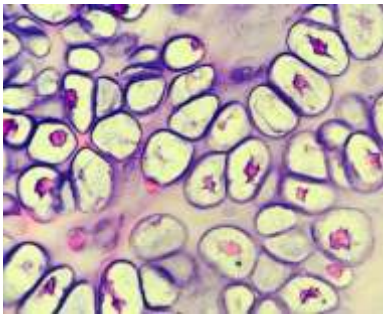

Figure 1: Chondroma Showing Cartilaginous Matrix with Scattered Uniform Appearing Chondrocytes in Lacunae (H & E stain, 400x magnification)

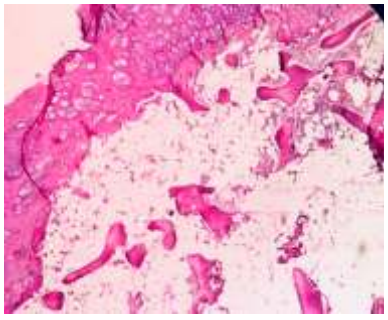

Figure 2: Exostosis (Osteochondroma) Showing a Cartilaginous Cap with Maturation into Trabecular Bone and Underlying Hematopoietic Marrow (H & E stain, 100x magnification)

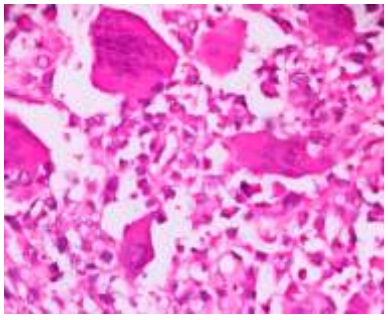

Figure 3: Giant Cell Tumor (Osteoclastoma). Numerous Multinucleated Osteoclast Giant Cells Scattered in a Background of Uniform Mononuclear cells (H & E stain, 200x magnification)

malignant tumors.

Metastatic (secondary) bone tumors comprised of 4(14.81%) cases, of which 2 were metastatic deposits of renal cell carcinoma (Figure 8), one presented as a pathological fracture in the femoral neck & one as a jaw tumor. One case of a rib tumor was a metastasis from breast cancer and one case of jaw tumor was metastases from cancer of the prostate (Table 2).

Regarding age distribution, patients with osteogenic sarcoma ranged from 5 to 54 years of age. Nine out of 14(64.29%) cases of osteogenic sarcoma were seen in children in the age range of 5 to 16 years. Chondrosarcoma occurred between 41 to 67 years of age and the 3 cases of Ewing sarcoma family of tumors were seen in the age range 7 to 15 years. A single case of chordoma was reported in the sacrum in a 32-year-old male patient. All 4 cases of metastatic deposits were above 60 years of age.

Out of a total of 4 cases of osteogenic sarcoma, 6 were of the telangiectatic type, 3 were chondroblastic, 1 was a small cell variant and the rest were the conventional subtypes.

Expression of the novel immunohistochemical nuclear stain SATB2 was studied in 14 suspected cases of osteogenic sarcoma. Twelve (87.71%) cases showed strong brownish-black nuclear positivity whereas 2 showed weak nuclear positivity (Figure 9).

Table 1: Histological Categorization and Frequency of Benign and Malignant Bone Tumors

Histological Subtype	Frequency	Percentage (%)
Benign Bone Tumors (n=56) 67.46%		
Exostosis (Osteochondroma)	24	42.85%
Giant Cell Tumor	16	28.57%
Chondroma	7	12.5%
Aneurysmal Bone Cyst	4	7.14%
Fibrous Histiocytoma	3	5.35%
Chondroblastoma	1	1.78%
Chondromyxoid Fibroma	1	1.78%
Total Benign Tumors	**56**	**100%**
Malignant Bone Tumors (n=27) 32.53%		
Osteogenic Sarcoma (Primary)	14	51.85%
Chondrosarcoma (Primary)	5	18.51%
Ewing Sarcoma Family of Tumors/ESFT (Primary)	3	11.11%
Chordoma (Primary)	1	3.70%
Metastatic	4	14.81%
Total Malignant Tumors	**27**	**100%**

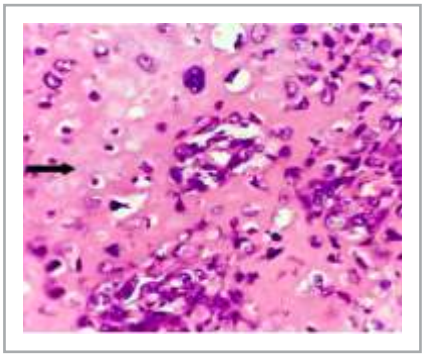

Figure 4: Osteogenic Sarcoma with a Lacelike Unmineralized Osteoid Matrix (Arrows) Deposited between Malignant Tumor Cells (H & E stain, 200x magnification)

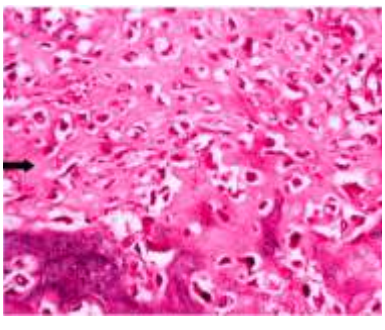

Figure 5: Chondroblastic Variant of Osteogenic Sarcoma Showing Abundant Osteoid (Arrows) and Chondroid Differentiation (H & E stain, 100x magnification)

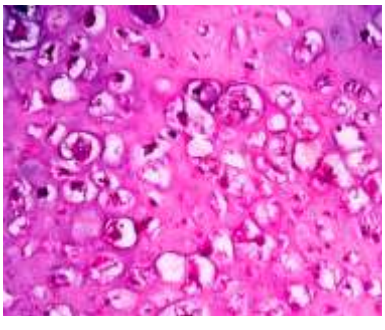

Figure 6: Chondrosarcoma (Grade 3) Showing Hyaline Cartilaginous Matrix with Admixed many Bizarre Looking Anaplastic Chondrocytes (H & E stain, 200x magnification)

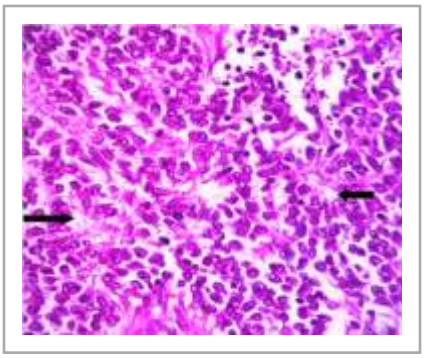

Figure 7: Ewing Sarcoma Family of Tumors (ESFT) Comprising of Small Round Blue Cells Arranged in True Rosettes and Perivascular Peudorosettes (Arrow) (H & E stain, 400x magnification)

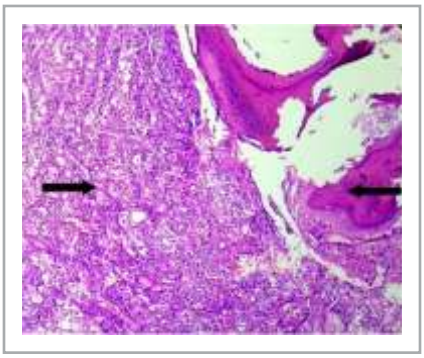

Figure 8: Metastatic Renal Cell Carcinoma (Arrow on the left side) in Bone (Arrow on the right side) (H & E stain, 100x magnification)

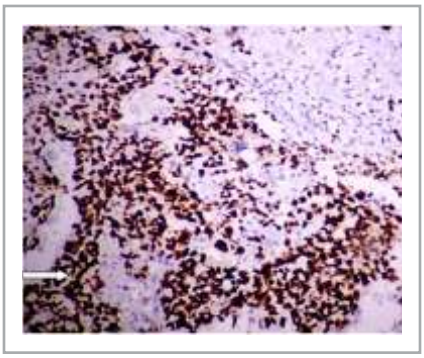

Figure 9: The Novel Immunohistochemical Stain SATB2 Showing Positive Nuclear Staining (Arrow) in Osteogenic Sarcoma (IHC stain, 100x magnification)

Table 2: Distribution of Bone Tumors According to Site

Benign	Phalanx	Sacrum	Humerus	Radius	Ulna	Femur	Tibia	Rib	Scapula	Jaw	Total
Osteochondroma			13			5	5		1		**24**
Giant cell Tumor			3	2	1	7	3				**16**
Enchondroma	2		1			1	3				**7**
Aneurysmal Bone Cyst			1	1	2						**4**
Benign Fibrous Histiocytoma			2	1							**3**
Chondromyxoid fibroma					1						**1**
Chondroblastoma							1				**1**
Malignant											
Osteogenic sarcoma			3	1	2	5	3				**14**
Chondrosarcoma					1	2		1		1	**5**
Ewing Sarcoma Family of Tumors (ESFT)			1	2							**3**
Chordoma		1									**1**
Metastatic						1		1		2	**4**
Total	2 (2.4%)	1 (1.2%)	24 (28.9%)	7 (8.4%)	7 (8.4%)	21 (25.3%)	15 (18%)	3 (3.6%)	1 (1.2%)	3 (3.6%)	83

DISCUSSION

Primary bone tumors comprise only 0.5% of the total world cancer incidence.[2] The diverse spectrum of bone lesions makes accurate diagnosis a challenging task. To establish an accurate diagnosis, a team approach is mandatory, taking into account all relevant clinical data, age, radiological studies, surgical notes & biopsy. Histopathological diagnosis helps in planning appropriate treatment protocols and to estimate the prognosis in individual cases.[7] In the present study, a total of 83 bone tumors were reported out of which there were 56 benign tumors (67.46%) and 27(32.53%) malignant tumors giving a ratio of almost 2:1 which concludes that the benign bone tumors far outnumber the malignant tumors. A study carried out in Iraq at the Kerbela University, Alwasiti Teaching Hospital from November 2015 to July 2017, concluded that out of a sample size of 119, benign cases reported were 100(84%) and malignant cases were 19(16%).[4]

In the present study, the most common benign bone tumor encountered was osteochondroma (42.85%), commonly known as exostosis followed by the giant cell tumor (28.57%), and chondroma (12.5%). The most common malignant tumor reported was osteogenic sarcoma (51.85%) and chondrosarcoma (18.51%). A study carried out in Mexico at a large

referral center in 2008, supports similar results with the most common benign tumors being osteochondroma (43.7%), followed by giant cell tumor (14.6%) and enchondroma (10.1%). Similarly, in the same study, the most common malignant tumor reported was osteosarcoma (46.6%) followed by chondrosarcoma (8.7%).[10] In a research conducted at a Medical College in Himmatnagar Gujrat, India in 2016, the most common benign bone tumors reported were osteochondroma & giant cell tumor and the most common malignant bone tumor was osteogenic sarcoma followed by chondrosarcoma.[11] However, in a study carried out in Patil Medical College in Pune, India in 2017, the most common benign tumor reported was giant cell tumor (30%).[7]

In the present study, the mean age of the patients presented with bone tumors was found to be 23±3.1 years with the youngest and the oldest patients being 5 and 67 years, respectively. Sixty one (58.7%) patients were males and 43(41.3%) were females. Similarly, in a study carried out in Mexico, the tumors affecting males were 53.7%, and 46.3% of females were diagnosed with bone tumors.[10]

In the present study, the most common bone involved was the humerus (28.9%) followed by the femur (25.3%). In a study carried out in Beijing, China in 2015, the most common bone involved was femur (37.7%) followed by tibia (20.1%) and humerus (8.4%).[12] Similarly, in another research conducted at Regional Institute of Medical Sciences, Imphal, Manipur India in 2013, the most common bones of involvement were femur (30.6%) and tibia (29%).[1]

The most common variety of osteogenic sarcoma found in our study was telangiectatic osteogenic sarcoma. Telangiectatic osteogenic sarcoma is a rare kind of osteogenic sarcoma comprising 2-12% of all cases of osteogenic sarcoma. They tend to behave more aggressively than conventional osteogenic sarcoma. This variant causes diagnostic difficulty in differentiating it from the aneurysmal bone cyst and cavernous hemangioma thus the use of SATB2 immunostain helps in demonstrating malignant osteoid.[13] The small cell variant of osteogenic sarcoma also causes diagnostic confusion with lymphoma and Ewing sarcoma family of tumors.[8]

Immunohistochemical stain SATB2 helps in confirming the diagnosis in this scenario as it highlights the osteoid matrix. The osteoblastic and small cell subtypes express SATB2 more intensely than other histological types.[14]

We found 4 cases of metastatic bone tumors, 2 from renal cell carcinoma, 1 from carcinoma breast, and 1 from prostatic carcinoma. Secondary bone tumors usually come from the respiratory system, gastrointestinal tract, prostate, breast, kidney, and liver. The axial skeleton because of its rich content of red marrow, has a predilection for harbouring metastatic deposits than the appendicular skeleton.[7] Carcinomas have a much high tendency to metastasize to the bone than sarcomas.[2] In a study carried out in near east population in Beirut, Lebanon, the most commonly metastasizing tumors to the bone were from breast, lung, and thyroid origin.[15]

In the present study, SATB2 was performed in suspected cases of osteogenic sarcoma. Out of 14(51.85%) cases of osteogenic sarcoma, 12(85.71%) showed positive nuclear staining. The diagnosis of osteosarcoma can pose certain diagnostic challenges, particularly in those cases when no convincing bone or osteoid matrix is identified especially in limited small biopsy samples to differentiate between hyalinized collagen & osteoid. In these situations, SATB2 has shown to be a reliable diagnostic marker for osteoblastic differentiation.[9]

In a study by Machado et al., the value of SATB2 in osteogenic sarcoma was evaluated and it was found to be reactive in 90.4% cases of conventional osteogenic sarcoma, 87.5% of small cell variant of osteogenic sarcoma, 91.3% of osteoblastic osteogenic sarcoma, and in all cases of chondroblastic and parosteal osteogenic sarcoma. However, osteoblastic and small cell variants showed stronger staining than other histological types.[8] In another study carried out in 2016, 45 out of 48(94%) cases of osteogenic sarcoma were immunoreactive for SATB2.[16] The results of SATB2 positivity of these different studies are very similar to the present study with 85.71% positivity.

As bone tumors are rare, most of the pathologists have difficulty in diagnosing them. Hence it is of utmost importance to have the relevant clinical data, age, gender, anatomical sites, and radiographic findings. Immunohistochemistry has a limited role in the diagnosis of bone tumors however new research is constantly being done in the field of molecular pathology for the diagnosis of bone tumors.

CONCLUSION

Osteochondroma is the commonest benign bone tumor and osteosarcoma is the most frequent malignant bone tumor. The immunohistochemical stain SATB2 proves to be a very effective tool in the diagnosis of osteosarcoma. It is a novel marker for bone tumors with osteoblastic differentiation and may help to distinguish osteogenic sarcoma from its mimickers like Ewing sarcoma family of tumors, lymphomas, aneurysmal bone cyst, & chondrosarcoma but biopsy & histopathological evaluation still remains the gold standard for accurate diagnosis of bone tumors.

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Original Article

Appraisal of Frequency of Malnutrition Indices and Relationship with Demographic Features among School Children

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ABSTRACT

Objective: To assess the frequency of malnutrition indices in the study population and to determine the relationship between different demographic features and nutritional status.

Methodology: This cross-sectional study was conducted at a school located in a rural area of Lahore from November 15, 2019, till April 30, 2020. A self-designed proforma registered the sample population. A total of 564 children, from a single school, were included. Centre for Disease Control (CDC) growth charts were used to record the centiles and Z-scores of the children. Cole classification was used to assess grading thinness/wasting. Hospital ethical committee approval was obtained. Informed consent was taken from parents of children before collecting data. Statistical Package for Social Sciences version 23 was used for data analysis.

Results: A total of 564 children were enrolled in the study. Among them, 49.6% were females, and 50.4% males. Moderate stunting was observed in 65(11.5%), and severe stunting in 13(2.3%). Body mass index (BMI) of less than 5th percentile was seen in 211(37.4%) cases. World Health Organization (WHO) weight for height Z-score, applicable to 191 students, placed 33(17.3%) as moderately undernourished and 17(8.9%) as severely undernourished. Normal BMI was observed in 342(60.64%) students, while overweight and obese were 8(1.42%) and 3(0.53%), respectively. The children were mildly 122(34%), moderately 87(15.4%), and severely 43(7.6%) underweight. Cole classification graded mild, moderate, and severe thinness in 165(29.3%), 102(18.1%), and 135(6.2%) children respectively. When we compared various malnutrition indices with population characteristics, only a significant association was found between the number of siblings and the degree of stunting (p-value=0.04).

Conclusion: Our study showed a significant number of undernourished children. As malnutrition has a substantial ill-effect on child health, there is a need to allocate more funds and resources to school going children to prevent malnutrition.

Keywords: *Malnutrition. Stunting. Underweight. Child health.*

INTRODUCTION

Pakistan is one of the countries with the highest pediatric mortality rate. According to the World Health Organization (WHO), malnutrition accounts for 54% of children throughout the world, in a total of about 1 million children.[1] The nutritional status of children in Pakistan is very poor and a significant number of children under 5 years of age are stunted. Pakistan has a high prevalence of child malnutrition as compared to other developing counties.[2] Almost half of the mortality in children around the globe is attributed to undernutrition. It is a well-recognized fact that macronutrient and micronutrient deficiencies lead to several health issues, not only affecting the general wellbeing and immunity of a child but also their development and intelligence.[3] This state of undernutrition leads to increased health problems in affected children, lowers their academic performance, and decline in overall productivity. The process is detrimental not only to the child himself/herself but also to the family and the nation as a whole. Malnutrition was found to be associated with impaired gut-barrier function, lowered exocrine secretion of protective substances, decreased level of plasma complement, atrophy of lymphatic tissue, and reduced delayed and humoral immunity.[4]

The regional research work shows a convincing relationship between various demographic features for the assessment of undernutrition and a higher risk of morbidity due to infections. The children with lower body mass index for age and having wasting had a higher number of infections. In a study from Bangladesh, school going girls from rural areas were more undernourished when compared to rural boys and urban children.[5] The local studies from different areas in the country have also shown the children with a varying magnitude of undernutrition, wasting, and stunting.

Data regarding the prevalence of malnutrition in Pakistan is limited mainly to children <5 years of age. There are very few publications evaluating malnutrition in school going children. There is always a search for demographic features that can be related to or are predictors of malnutrition. Therefore, we conducted this study to find a relation between malnutrition indices and demographic data to elucidate the burden of malnutrition in our school going children and to find the risk factors causing this effect, so that health can be improved in this often neglected echelon of our society.

METHODOLOGY

This study was conducted at a school located in a rural area of Kahna, Lahore from November 15, 2019, till

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April 30, 2020. The study was approved by the ethical committee of the institution. The cross-sectional study, using a convenience sampling technique, was done on 564 students during the study period. All children between 4-15 years of both genders were included. Children having congenital diseases or chronic illnesses were excluded. A proforma was designed to register the child's name, age, sex, weight, height, number of siblings, parents' education level, and monthly income of the family. Before enrollment in the study, informed consent was obtained from children's parents and school administration. Ethical approval was obtained from the hospital ethics committee to conduct the study.

Anthropometric measurements like height, weight, body mass index (BMI), stunting, weight for height Z-score, underweight & thinness were done. Children's height in centimeters was made by stadiometer. Weight in kilograms was measured by making the child stand on an electronic weighing scale wearing thin clothes and without shoes. Zero error of the machine was adjusted before each measurement.

Body mass index (BMI) was calculated using the formula; Weight in kilograms/(Height in meters)^2. Centiles and Z-scores were recorded using CDC growth charts. To calculate the weight for height percentile and Z-score, the WHO criterion was employed. Z-score for BMI was calculated using the LMS method, which summarizes the distribution of BMI by age and sex in terms of three curves called L (lambda), M (mu), and S (sigma)

$Z=[(BMI/M)L-1]/(L\times S)$[6]

Following classifications were used for recording malnutrition:

1. BMI: Low BMI: Less than 5th percentile
Normal BMI: Between 5th and 84th percentile
Overweight: ≥85th to 94th percentile
Obese: ≥95th percentile

2. Stunting: Moderate stunting is defined as height for age less than or equal to -2 Z-score and severe stunting is a height for age less than or equal to -3 Z-score.

3. Weight for height Z-score: In children whose height was less than 120 cm, WHO weight for height Z-score charts was utilized.
Moderate undernutrition: Z-score ≤-2
Severe undernutrition: Z-score ≤-3

4. Underweight:Mildly underweight: Z-score ≤1
Moderately underweight: Z-score ≤2
Severely underweight: Z-score ≤ 3[6]

5. Thinness: Cole classification was used for undernutrition.
Mild thinness: BMI Z-score less than -1
Moderate thinness: BMI Z-score less than -2
Severe thinness: BMI Z-score less than -3[7]

STATISTICAL ANALYSIS

Data was entered on Statistical Package for the Social Sciences (SPSS) version 23. Frequencies and percentages were used to describe demographic data and malnutrition indices. Chi-square was used to compare demographic features with malnutrition indices. The p-value of ≤0.05 was considered significant.

RESULTS

In the study, 564 patients were enrolled, of which 280(49.6%) were females and 284(50.4%) were males. The majority of the children (69%) were between 6-10 years, 42(7.4%) were less than 6 years and 133(23.6%) were >11 years. The mean age was 8.76±2.3 years. Demographic data is depicted in Table 1. Moderate stunting was present in 11.5% and severe stunting in 2.3% of the children (Figure 1). The majority of the students, 342(60.64%) were of normal BMI, while overweight and obese were 8(1.42%) and 3(0.53%) respectively (Figure 2).

Table 1: Demographic Distribution of Study Population (n=564)

Demographic Variables	Findings			
Age in years	**Mean±SD**	**Minimum**	**Maximum**	
	8.76±2.3	4	15	
Number of Siblings	**<3**	**3-5**	**>5**	
	60(10.6%)	287(50.9%)	217(38.5%)	
Educational Status of the Father	**Illiterate**	**Primary/Middle**	**Matriculation**	**Graduation and Above**
	204(36.2%)	238(42.2%)	83(14.7%)	39(6.9%)
Educational Status of the Mother	**Illiterate**	**Primary/Middle**	**Matriculation**	**Graduation and Above**
	334(59.2%)	167(29.6%)	47(8.3%)	16(2.8%)
Income per Month (Rupees)	**<20000**	**20000-50000**	**>50000**	
	439(77.8)	115(20.4%)	10(1.8%)	

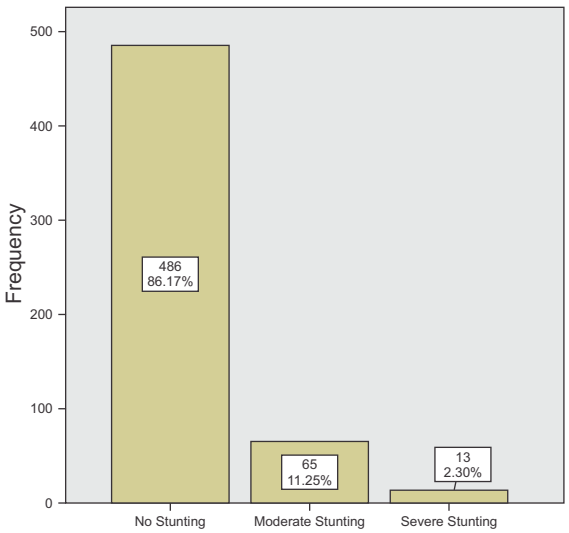

Figure 1: Stunting Frequency in Study Population (n=564)

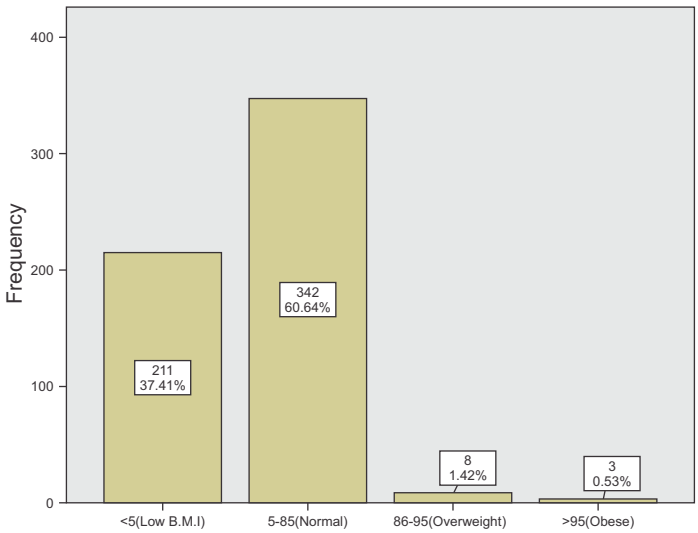

Figure 2: Study Population Distribution According to BMI Centiles (n=564)

World Health Organization weight for height Z-score is an important parameter in evaluating undernutrition in developing countries. It is for children less than 5 years or who have a height of less than 120 cm. So, a separate group of 191 participants with a height of less than 120cm, was made. In this group, weight for height Z-score revealed that 141(73.8%) were either normally nourished or mildly undernourished, as WHO weight for height classification does not include mild malnutrition definition. In this group, 33(17.3%) were moderately undernourished, while 17(8.9%) were severely undernourished. Taking into account, another system of classification where weight is measured on a graph against height and then Z-score is calculated, a total of 322(57%) were underweight while 242(43%) were of normal weight.

According to Cole classification, thinness was observed in 262(46.4%) children. Mild, moderate, and severe thinness were present in 165(29.3%), 102(18.1%), and 35(6.2%) of the study group, respectively.

Data was compared and evaluated for gender, family income, age distribution, number of siblings, and the educational status of the parents, with the malnutrition indices. The only significant correlation found was between the number of siblings and stunting (p-value=0.04). Table 2 shows the correlation of demographic variables with malnutrition indices.

Table 2: Relationship between Demographic Variables and Malnutrition Indices

Demographic Variables	Malnutrition Indices			
	Stunting n=564 p-value	BMI n=564 p-value	Thinness n=564 p-value	Under nutrition (weight for height) n=191 p-value
Gender	0.36	0.92	0.66	0.42
Age Range	0.48	0.96	0.96	0.10
Family Income	0.28	0.26	0.47	0.91
Number of Siblings	0.041*	0.14	0.96	0.35
Father's Education	0.45	0.22	0.79	0.2
Mother's Education	0.8	0.14	0.14	1.62

*Significant p-value

DISCUSSION

In our study, 11.5% children were moderately and 2.3% were severely stunted. Stunting indicates chronic malnutrition. Our study shows a high number of stunted children, though severe stunting was observed in a very small number of cases. In a study conducted in Abottabad, 10% of the study population was stunted while severe stunting was present in only 0.1%.[8] In a study conducted in Nigeria, 348 children from both the private and the public sector showed that 7(0.8%) children were stunted.[9] The difference could be because the current study population includes one school located in a poor area while cited researchers included the children from both poor and rich strata of the community. In a report from war-ravaged South Sudan, that included a hundred and nine students from a school, 15% were stunted though 73% were having wasting (BMI less than 5%).[10] Our results are in contrast to what has been reported in a Multiple Indicators Cluster Survey (MICS-2014) conducted in Punjab, showing a frequency of 37% stunting.[11] Another research article from Sindh, revealed stunting in under age 5 children at 35%.[12] As these researches included several studies, so results are more representative of the situation in Pakistan. One reason for lower rates of stunting in our study could be the WHO classification used for categorizing stunting which does not address mild cases.

Taking into account WHO criteria of weight for height Z-score, for classifying malnutrition, we made a separate group of children whose height was less than 120cm. We found 17.3% of children were moderately undernourished and 8.9% were severely undernourished. In our study, we found that unlike stunting, BMI <5 percentiles was present in a much higher number of study population i.e., 211(37.4%). Our data showed mild (34%), moderate (15.4%), and severe (7.6%) underweight school children. In a survey conducted in a primary school setting in Zambia, 9.7% were wasted, 24.5% were stunted and 14.9% were underweight.[13] On the contrary, we found 37.4% of children presented with wasting, and 13.8% were stunted. While taking into account, underweight children, our data reveals that a higher number (57%) of children were underweight as compared to the Zambian study where only 14.9% of the study population showed this feature.[13] The difference could be due to the difference in the definition and categorization of malnutrition.

In our study population, only 1.4% of students were overweight and 0.53% obese. In a research article from Nigeria, overweight and obesity were observed in 8.5% and 4.1% of children respectively.[9] It was much higher as compared to our data, probably because the Nigerian study included children from both the private and the public sectors, unlike our study population where the majority belonged to the poor class. In a study conducted in the primary schools in Abbottabad from varying socioeconomic groups, the underweight population varied from 45-51% with a percentage of severe malnutrition being 2.8%.[8] The data collected from affluent areas of Pakistan, showed that the majority of the children had normal BMI.[14]

In our data analysis, the only parameter found to be significantly associated with stunting was the number of siblings (p-value=0.04), while we found that age, gender, family income, and maternal or father education did not have a significant correlation with the degree of malnutrition. It is contrary to other publications. A study showed that the consumption of food that is inadequate in required calories and less than four varieties of food groups consumed by the children, are important predictors of malnutrition.[14] A research article concluded that issues of son preference/daughter aversion also affect child malnutrition in South Asia.[15]

No significance was observed for the number of children and malnutrition in a study from Iran.[16] In various Pakistani publications, uneducated or less educated mothers put their children at risk of developing malnutrition. In a recent study conducted in Pakistan, a higher paternal educational status (p=0.008) and maternal educational status (p=0.011) were found to be significantly associated with normal child nutritional status.[17] The difference is because a majority of the parents in our study population belonged to the poor education status.

In an article from South Africa, severe thinness was more prevalent in younger children as compared to older children where mild wasting/thinness was a chief finding.[18] In our study, overall thinness, according to BMI Z-score, was found in 53.6% of children, moderate thinness in 18%, and severe thinness in 6.1% of participants. Another article using Cole classification for undernutrition, reported overall thinness of 61%. Severe thinness was seen more among the girls than the boys in this study (18.7% vs. 17.6%).[19] The results are comparable to our study.

Early childhood is a critical period of life during which parameters like good nutrition, improved child care, improved protection, and stimulation can have maximum impact on growth including brain development. Early interventions can even affect future earnings. Research has shown that preventing early childhood undernutrition improves earning by 20% in later life.[20]

CONCLUSION

Malnutrition is a significant health problem. Our study showed a high number of school-aged children suffering from undernutrition, highlighting the importance of inducting measures in the national policies to overcome this issue. Considering the impact

of malnutrition on child health, child psychology, development, and its influence on overall childhood morbidity and mortality, it is obligatory to divert a larger portion of our national resources to eradicate childhood malnutrition.

LIMITATIONS

The study included children from one school, representing a limited study population. There should be a study inducting children from several schools from different areas of the town to strengthen and to generalize the results. No formula was used to calculate the sample size to increase the strength of the study.

RECOMMENDATIONS

There is a need, on a priority basis, for more sound research work to include school going children from all economic strata to elucidate malnutrition and various factors affecting this condition. Further publications, assessing the impact of foodstuff consumed, diseases, vaccines, and other such parameters on the nutrition of school going children, should be emphasized. Parents should be educated about malnutrition and its effects to prevent further complications.

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Original Article

Current Laboratory Practices for the Fabrication of All-Ceramic Restorations in Lahore

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ABSTRACT

Objective: To find out the frequency of use of ceramic materials and restoration techniques amongst dental laboratories of Lahore.

Methodology: This was a cross-sectional study conducted in dental laboratories of Lahore using a convenient sampling technique. The questionnaire was distributed in 20 dental laboratories in Lahore and the participants were asked to answer the survey regarding their preferences, practices, and choice of techniques employed for the fabrication of all-ceramic restorations.

Results: Nineteen dental laboratories gave a response to the questionnaire. The results of this study indicate an equal preference (36.8%) of computer-aided design and manufacturing (CAD/CAM) and pressable ceramics techniques for all-ceramic crowns. Zirconia is the material of choice due to its excellent aesthetic property and color matching ability amongst 10(52.6%) dental laboratories. Glass-based lithium disilicate ceramics are most commonly used due to their durability amongst 8(42.1%) laboratories.

Conclusion: Computer-aided design and manufacturing (CAD/CAM) and pressable ceramics are the most preferred fabrication techniques of all-ceramic crowns, while zirconia-based materials are the most popular amongst laboratory technicians.

Keywords: *Ceramic restorations. Zirconia. Glass based lithium disilicate ceramics. Pressable ceramics. CAD/CAM.*

INTRODUCTION

With the advent of newer materials and technologies, traditional metal-ceramic or porcelain fused to metal (PFM) crowns have been replaced with all-ceramic restorations worldwide. The major problems of PFM crowns were the chipping of porcelain and compromised aesthetics due to metal margins. All-ceramic restorations were very expensive when first introduced but nowadays they are used in almost all dental laboratories. There is also a growing trend for using all-ceramic crowns amongst contemporary dentists.[1]

The primary indication of using an all-ceramic restoration is favorable aesthetics.[2] All-ceramic materials mimic the optical properties of teeth, such as translucency. As regard to cost-effectiveness, these are also the material of choice.[3] The main shortcoming of all-ceramic restorations was low mechanical strength, which restricted their use to the anterior sextant and single unit prostheses.[4]

The all-ceramic crown in dentistry was introduced by Land in 1903. These crowns exhibited good aesthetics but compromised mechanical properties. In 1960s, feldspathic porcelain was used as a veneer over a metal crown. However, it could not be used for the all-ceramic crown as it required a tougher core ceramic or metal foil coping.[5,6]

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Improvements in the composition and fabrication techniques led to the introduction of all-ceramic anterior and posterior restorations. In 1965, McLean and Hughs introduced aluminous porcelain for the fabrication of porcelain jacket crowns.[5] Due to the opacity of aluminous porcelain, feldspathic material was preferred. Afterward, leucite-reinforced and lithium disilicate-reinforced glass-ceramics were introduced.[6,7]

Clinical performance results revealed that there was a problem of chipping of veneered porcelain; hence, monolithic restorations were introduced. Continuous progression led to the emergence of zirconia as a strong material for all-ceramic restorations. With the advent of translucent zirconia, it could be used for anterior teeth as well.[8]

The success and survival rate of an all-ceramic restoration depends upon many factors, such as the type of material used, the fabrication technique employed, and its location in the oral cavity. According to Aldegheishem et al., the survival rate of all-ceramic restorations ranges from 88-100% after 2-5 years and 84-97% after 5-14 years.[9] Another study by Sailer et al., investigated the longevity of different types of ceramic materials. It was concluded that alumina (96%) and zirconia (92.1%) crowns were far more successful in both anterior and posterior regions.[9,10]

Dental laboratory technicians play an important role in the provision of good quality restorations. During the fabrication of an all-ceramic crown, a laboratory technician should know different materials and their fabrication techniques. To improve the aesthetics of the restorations, they should be aware of the optical properties of different all-ceramic materials. Thus, the goal of this study was to find out the frequency of use of ceramic materials and restoration techniques amongst

dental laboratories of Lahore.

METHODOLOGY

A descriptive cross-sectional study was conducted, following approval from the ethical review committee of Fatima Memorial Hospital (FMH) College of Dentistry, Lahore. Informed consent was taken from every participant ensuring the confidentiality of data. The questionnaire was distributed among 20 private dental laboratories in Lahore, which incorporated the facilities for fabrication of all-ceramic restorations with computer-aided design and computer-aided manufacturing (CAD/CAM) equipment. Thus convenient sampling technique was employed. The participants were inquired about their preferences and practices regarding the choice of materials, properties of materials, fabrication techniques, ease of fabrication, durability, prescription, and preferences of the dentists. The questionnaire was developed by a team composed of subject specialists, the authors, a laboratory technician, and a biostatistician from FMH College of Dentistry, Lahore. After the development of a questionnaire, a pilot study was conducted, the questionnaire was validated and used in this study.

STATISTICAL ANALYSIS

For data analysis, all data was entered in Statistical Package for the Social Sciences (SPSS) version 23. Frequencies and percentages were calculated for the categorical data obtained from the questionnaires.

RESULTS

Out of the 20 forms distributed, 19 were filled, giving a response rate of 95%. The majority of dental technicians were fabricating all-ceramic crowns by pressable and CAD/CAM techniques (Figure 1). About 10(52.6%) technicians were using zirconia-based ceramics, 6(31.6%) were using glass-based

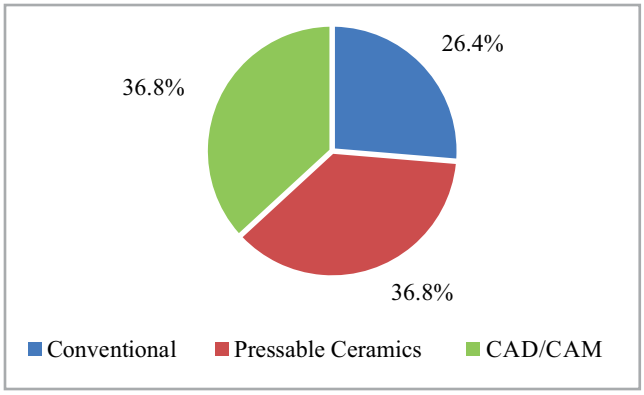

Figure 1: Percentage of Crown Fabrication Techniques used by Dental Technicians

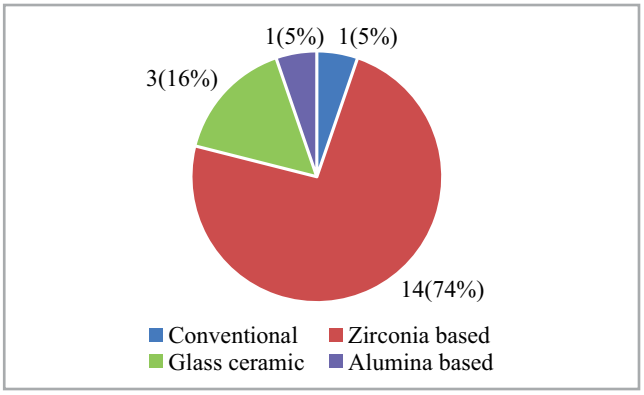

Figure 2: Frequencies of Dental Materials used by Dentists for All-ceramic Crown

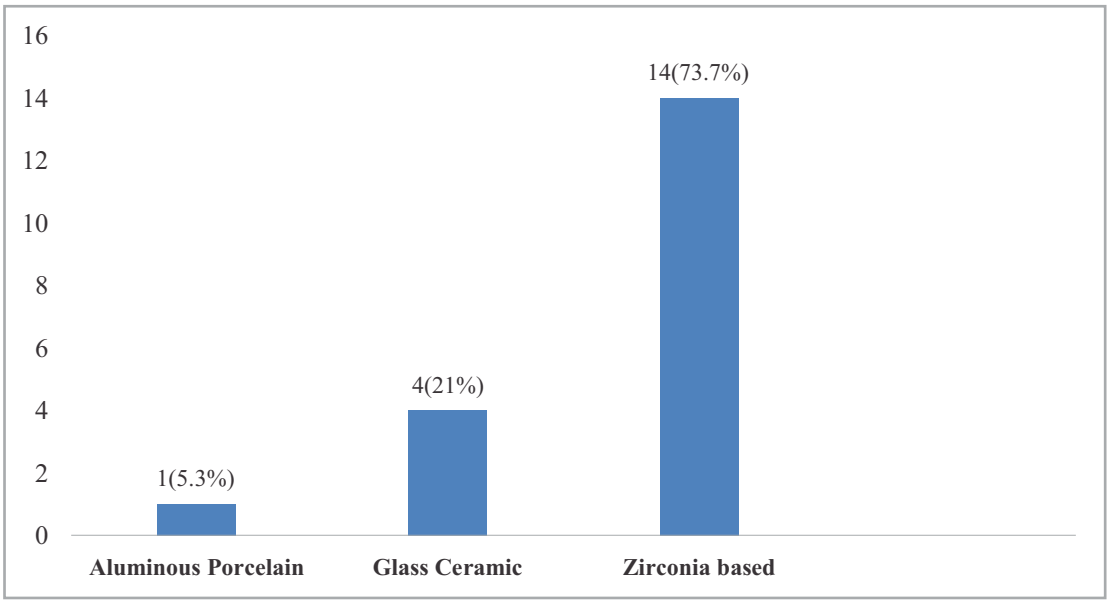

Figure 3: Frequencies & Percentages of Dental Materials used by Dental Technicians for All-ceramic Crown

ceramics, 2(10.5%) used alumina-based porcelain and 1(5.3%) was using lithium disilicate ceramics.

About 11(57.9%) dental technicians preferred zirconia because of its best optical properties, but this was a personal observation amongst them, followed by 7(36.8%) preferring glass reinforced ceramics and 1(5.3%) preferring aluminous ceramics.

Lithium disilicate (42%) was preferred by dental technicians for its durability, followed by zirconia (37%) and aluminous porcelain (21%). The choice of material and techniques used for fabrication were essentially done after consultation with the prescribing dentist in 16(84%) laboratories whereas 3(16%) laboratories constructed crowns without such approval. The frequency of dental materials preferably used by the dentist and the dental technicians are shown in Figures 2 and 3, respectively.

DISCUSSION

The popularity of all-ceramic restorations is increasing in Pakistan and they are now being frequently used by local dentists. Most of the contemporary dental laboratories are offering a variety of all-ceramic restorative options. The choice of the material varies with different clinical scenarios as well as the dentists' and technicians' preferences.

The selection of materials may be limited due to the unavailability of the required technology and technical expertise. The cost of the equipment and expenditures incurred often determine the fabrication technique. Recent studies have concluded that CAD/CAM technique is the most widely used method of fabrication for all-ceramic restorations.[11-13] Shah et al. deduced that pressable ceramics were most commonly used by dental technicians for masking discolored anterior teeth.[7] This study indicates an equal usage of CAD/CAM and pressable ceramics in the dental laboratories of Lahore.

Over a period of time, aesthetic demands of the patients led to innovations in all-ceramic technology. New materials were developed to improve the optical as well as mechanical properties of all-ceramic restorations.[14] Glass-based ceramics e.g. lithium disilicate exhibited excellent aesthetics similar to that of the natural dentition. However, their mechanical strength was compromised. Initially, zirconia was not preferred for anterior restoration because of its opacity.[15] However, with the advent of translucent monolithic zirconia it became possible to fabricate anterior crowns with this material as it provided adequate strength and aesthetic properties.[16] The current study also concluded that most of the dental technicians preferred zirconia (57.9%) due to its properties.

Durability is an important criterion for the selection of a restoration and is dependent on the type of material, restorative procedure and location of restoration in the mouth. Moreover, the clinical performance can be predicted by the survival rate and stability of restoration over a period of time. Lithium disilicate is the most economical material and has good longevity. Our study concluded that lithium disilicate was the most preferred material (42.1%) based on restoration longevity values. A study by Mobilio et al., reported a high survival rate (97.7%) of lithium disilicate crowns after 4 years.[17] Another clinical data exhibited 95% survival rate after 4 years.[18]

Our results showed that 84% of technicians received instructions from their dentists regarding the choice of material and fabrication techniques. Another study showed that about 95% of technicians take instructions from the dentist through work authorization forms for communication about the restorative work.[19] Effective communication between dentist and laboratory technician is very important for the success of all-ceramic restorations.[20,21]

Zirconia is the material of choice for dental technicians and dentists. Its high strength combined with adequate aesthetics led to its use for posterior restorations.[22] Teichmann et al. analyzed different all-ceramic systems and concluded that zirconia can be a good option for posterior crowns due to its strength.[6] This study concluded that 73.3% of dentists preferred zirconia for all-ceramic restorations, while 73.7% of technicians preferred zirconia for posterior crowns.

The evolution of materials and techniques has led to a remarkable increase in the fabrication of all-ceramic restorations. Advancement in fabrication techniques resulted in the popularity of CAD/CAM technology in Lahore as compared to other conventional techniques because of the low cost of restoration and time saving factor. However, the initial installation of the CAD/CAM apparatus requires more investment, so the government should take initiative to help in the installation of these machines in dental hospitals. There should be more awareness among dental technicians about the use of this technology.

CONCLUSION

The high cost of CAD/CAM equipment and technique has deterred most laboratories from using this technology. Pressable ceramics offer a good alternative due to aesthetics and economic viability. Hence, CAD/CAM and pressable ceramics are the most preferred fabrication techniques for all-ceramic crowns, while zirconia-based materials are the most popular amongst laboratory technicians.

LIMITATIONS AND RECOMMENDATIONS

A major limitation of the study was that popular private laboratories were enrolled during the survey recording. Less commercialized private laboratories may have been missed in the city. This was a survey-based study

that took into account the preferences and practices in private laboratories and not the public ones. A follow-up study would be required to note the changes in practices, after conducting a continuing dental education session, for both the dentists and the dental technicians.

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Original Article

Serum Uric Acid and Homocysteine Levels as Predictors of Preeclampsia in Young Pregnant Women

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ABSTRACT

Objective: To find out the role of serum uric acid and homocysteine levels as predictors of preeclampsia in young pregnant women.

Methodology: A cross-sectional study was carried out at Lahore General Hospital from a period of May 2017 to February 2018. A total of 35 consented preeclamptic women in the 2nd and 3rd trimester of pregnancy (preeclamptic pregnant group) with an age range of 21-28 years were included in the study. Thirty consented age-matched pregnant women with no history of any disease were taken as normotensive pregnant controls. Women with a blood pressure of 140/90 mmHg and proteinuria were considered as preeclamptic pregnant females. Questionnaires based on age, gestational period, parity, etc. were filled by both groups. Blood samples of both patients and controls were collected and analyzed for the serum levels of uric acid and homocysteine. Collected data was analyzed by Statistical Package for the Social Sciences (SPSS) version 20.

Results: Mean age of preeclamptic pregnant females was 35±6 years with a mean BMI of 30 kg/m^2. Their mean blood pressure was 143.55/93.87 mmHg. The majority of the pregnant women included in the study belonged to a poor socioeconomic class with a history of obstetrical complications like abortions in previous pregnancies. Significantly increased levels of serum uric acid and serum homocysteine were observed in the preeclamptic pregnant group as compared to the normotensive pregnant control group.

Conclusion: Levels of serum uric acid and homocysteine are raised in preeclamptic pregnant females. Estimation of maternal hyperuricemia and hyperhomocysteinemia can be a good parameter for assessing the prognosis and management of patients with pregnancy induced hypertensive disorders including preeclampsia. Furthermore, these serum markers may play an important role in understanding the etiology and pathogenesis of preeclampsia.

Keywords: *Hyperuricemia. Hyperhomocysteinemia. Preeclampsia.*

INTRODUCTION

Preeclampsia is a medical illness that is defined as a combination of pregnancy induced hypertension and proteinuria commonly observed in the second trimester after twenty weeks of pregnancy. It may provoke vascular and metabolic changes and increases the risk of cardiovascular diseases, diabetes mellitus, and various renal problems at later ages.[1] The sequelae of preeclampsia usually start after the 20th week of pregnancy in previously normotensive women. It can result in a poor outcome for both the infant and the mother. Negligence about preeclampsia during pregnancy can lead to a grave condition termed eclampsia, which greatly increases the risk of perinatal mortality.[2] Worldwide prevalence rate of preeclampsia is roughly 7 to 15% which may increase with increasing age. It can affect any organ of the body and leads to morbidity and mortality in both the mother and the fetus.[3]

Uric acid is a major end product of purine metabolism. It has been illustrated by many studies that uric acid may be taken as a pathogenic marker in cases of preeclampsia and hypertensive disorders of pregnancy. Normally, during early pregnancy, serum uric acid level falls to 3 mg/dl or even low because of uricosuric effects of estrogen produced by the placenta and increased blood flow to kidneys. However, in later pregnancy, normally, a rise in serum uric acid is seen.[4] Additionally, increased production of uric acid due to the breakdown of trophoblast fetal cells combined with reduced clearance of uric acid results in hyperuricemia in pregnant women with preeclampsia. The increased levels of serum uric acid alter the production of nitric oxide in cells of vascular endothelium causing endothelial dysfunction, the release of cytokine, ischemia, and inflammation.[5] It can be taken as a useful biomarker for diagnosis and also has a contributory role in the pathogenesis of fetal and maternal manifestations of hypertensive disorders. Uric acid blocks vascular endothelial growth factor (VEGF) induced endothelial proliferation and directly blocks fetal angiogenesis leading to intrauterine growth retardation, kidney dysfunction of the fetus, and trophoblastic invasion of the placenta.[6]

Homocysteine is a thiol-containing amino acid and an intermediary demethylated metabolic derivative of sulfur-containing essential amino acid methionine. It has been observed in various studies that raised serum homocysteine levels are associated with an increased risk of dysfunction of endothelial cells and injury to the vascular tissue leading to atherosclerosis and occlusion of vessels due to the thromboembolic phenomenon. Normally, during pregnancy, mean serum homocysteine level decreases with the gestational age due to physiological response of pregnancy, rise in estrogen levels, hemodilution due to increased plasma volume, and increased demand for methionine by both

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the mother and the fetus. Physiologically, homocysteine metabolizes by two pathways: one is remethylation to methionine and the other is the synthesis of cysteine. It needs folate as a substrate and vitamin B_{12} as a coenzyme for these reactions.[7] The elevated levels of serum homocysteine in women with preeclampsia exists in early pregnancy and remain high till delivery. A rise in serum homocysteine, an intermediary metabolite during pregnancy is associated with an increased risk of complications like abruption of the placenta, preeclampsia, restricted growth of the fetus, and even loss of pregnancy and reduced renal function.[8]

Hyperuricemia may occur in association with hyperhomocysteinemia due to the alteration in the metabolic pathways. Besides, the increased levels of both of these serum parameters may cause dysfunction of endothelium that result in preeclampsia. However, the degree to which serum uric acid and homocysteine independently affect the function of the kidney and increase the risk of preeclampsia is still not known.[5]

Several serum markers for early detection of preeclampsia have been suggested but have not yet been proven. There is a need for serum markers which may serve as early predictors of preeclampsia and may help to protect both the fetus and the mother from severe consequences. The present study was planned to assess the levels of serum uric acid and homocysteine in two groups namely, preeclamptic pregnant patients and the normotensive control group, and to analyze the role of these two serum markers as early predictors of preeclampsia.

METHODOLOGY

This was a cross-sectional study and conducted at Lahore General Hospital from a period of May 2017 to February 2018. The study was ethically approved by the IRB committee of PGMI Institute Lahore. A convenient sampling technique was employed for the collection of data. The study population was divided into two groups. Thirty-five consented preeclamptic women in the 2nd and 3rd trimester of pregnancy with an age range between 21-28 years were taken as a preeclamptic pregnant group and thirty consented age-matched pregnant women with no history of any disease were taken as a normotensive pregnant control group. Preeclamptic pregnant females who were diagnosed at the time of admission and before the beginning of antihypertensive treatment were included in the study. Gestational age of women was estimated by the scan with preeclampsia in pregnancy index. Pregnant women with a blood pressure of 140/90 mmHg and 1+ proteinuria were considered as preeclamptic females. Pregnant women with any chronic problem like liver/renal dysfunction or a history of taking drugs were excluded from the study. The questionnaires based on age, gestational period, parity, etc., were filled by both the preeclamptic women and the controls.

Blood samples were collected from the antecubital veins of both groups. All the collected samples were sent to the laboratory within one hour of collection. These samples were subjected to centrifugation at 3000 rpm for 5-10 minutes and clear serum obtained was collected in a vial and stored at -2 to -8 degree centigrade. Elisa kit method was used to assess the serum homocysteine level. Serum uric acid level determination was done by the indirect equilibrium uricase method.

STATISTICAL ANALYSIS

Collected data was analyzed by Statistical Package for the Social Sciences (SPSS) version 20. Quantitative data was expressed as mean and standard deviation. Comparison of serum levels of uric acid and homocysteine between preeclamptic pregnant women and normotensive controls was performed by independent sample t-test. A p-value ≤0.05 was considered significant.

RESULTS

Demographic profiles of preeclamptic pregnant females were collected. It was observed that the mean age of pregnant females with preeclampsia was 35±6 years with a mean BMI of 30 kg/m^2. Their mean blood pressure was 143.55/93.87 mmHg. The average years of marriage were 3.11 years. Among the preeclamptic group, multigravidas were 11 with a parity of 3 while the rest were primiparas with a gestational age of 25 weeks. Most of the patients (85%) belonged to poor socioeconomic status and 14(40%) of the preeclamptic patients had a history of abortions.

Mean values of serum uric acid and homocysteine are shown in Table 1 & Figure 1. Serum levels of uric acid and homocysteine were compared in both the patients and the controls. It was observed that significantly

Table 1: Comparison of Serum Uric Acid and Homocysteine Levels in Study Subjects

Serum Markers	Pre-eclamptic Pregnant Females	Normotensive Pregnant Controls	p-value
Serum Uric acid (mg/dl)	6.16±1.54	3.68±0.67	0.001*
Serum Homocysteine (µmol/L)	20.09±3.45	6.35±2.39	0.05**

* Highly significant p-value=0.001 **Significant p-value=0.05

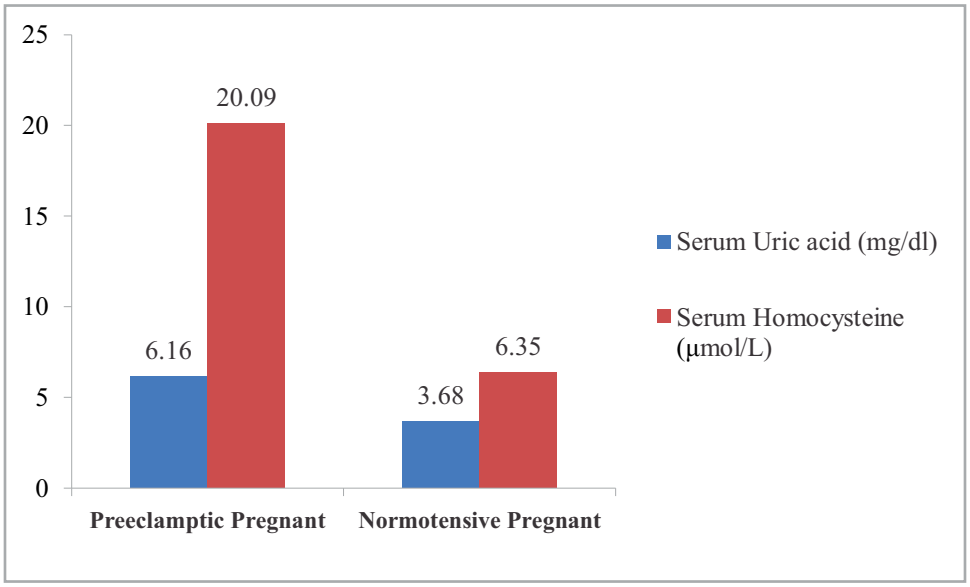

Figure 1: Comparison of Study Variables in Preeclamptic Pregnant Females and Controls

increased levels of serum uric acid and serum homocysteine were observed in patients as compared to the control group with a p-value of 0.001 and 0.05 respectively (Table 1).

DISCUSSION

Preeclampsia is a term used for the disorder related to blood pressure seen during pregnancy. It occurs at around 20 weeks of gestation. It has been observed by Kunwar et al., that preeclampsia is responsible for complicating 1% of all pregnancies.[9] According to Yelikar et al., pre-eclampsia affects 5-7% of all pregnancies and is mostly found in obese and elderly pregnant females.[10] Preeclampsia remains a great public health issue in both developing and the developed countries. Preeclampsia, still, is considered a major contributing factor of maternal and perinatal morbidity as the course of illness and its exact etiology remains unknown till today. Although endothelial dysfunction and associated vasospasm are considered as major contributing factors, however, recently other causes have also been suggested in this regard. The most important among those are hyperuricemia and hyperhomocysteinemia that have gained a lot of attention in the recent past and much work is currently been done to explore the role of these two serum markers in preeclampsia.[11]

Homocysteine is an intermediate metabolite of methionine, an essential amino acid and it has been postulated to be responsible for the production of oxidative stress and endothelial injury which may lead to preeclampsia. In a recent study by Kunwar et al., it has been observed that concentrations of both homocysteine and uric acid were elevated significantly in maternal plasma in patients with preeclampsia which was in accordance with our results.[9] Other studies also supported the hypothesis that preeclampsia may be associated with a higher concentration of homocysteine in maternal blood.[12,13] Until now, the exact etiology of raised serum homocysteine and uric acid levels in preeclampsia has not been figured out. A study conducted by Shahbazian et al., stated that by measuring the levels of serum homocysteine, folic acid, and vitamin B_{12}, women at risk for preeclampsia could be screened, but they failed to prove that use of folic acid and vitamin B_{12} in pregnant women could decrease the level of serum homocysteine.[14] Holmes et al., also supported this school of thought and suggested that continued folic acid supplementation during pregnancy lowers the risk of high homocysteine concentration in maternal serum during the third trimester.[15]

Our results are consistent with the results of a study by Noori et al., and another study by Maru et al., where a significant relationship was found between raised homocysteine and severity of preeclampsia.[12,16] The hypothesis was further strengthened by another study by Sun et al., which showed that hyper-homocysteinemia was caused by endothelial disruption of arteries that may lead to placental infarction or abruption resulting in repeated miscarriages, fetal growth retardation, and neurological dysfunctions.[17] All these observations and results were concluded by another study held in Pakistan that showed a significantly increased homocysteine levels were seen in preeclampsia in the second and third trimester of pregnancy.[18] In our present study, raised serum uric acid levels were significantly correlated with the preeclamptic pregnant patients. A study conducted by Yadav et al., concluded that serum uric acid and serum creatinine level increase significantly in preeclampsia

in comparison to the controls.[19] A study held in India in 2019 concluded that increased uric acid levels can affect fetal health and have adverse effects on fetal outcome.[20] Another study observed that an increase in serum uric acid level is a major cause for pregnancy induced hypertension and uric acid can be used as a marker to predict the severity of this condition.[21] All the above mentioned studies and their results are comparable with the results found in our present study, strengthening and supporting the correlation of increased serum uric acid and homocysteine levels with preeclampsia. Further research is needed to describe the relationship between serum concentrations of uric acid and homocysteine, and the severity of preeclampsia.

CONCLUSION

Levels of serum uric acid and homocysteine are raised in preeclamptic in pregnant females. Estimation of maternal hyperuricemia and hyperhomocysteinemia can be a good parameter for assessing the prognosis and management of patients with pregnancy induced hypertensive disorders including preeclampsia. Furthermore, these serum markers may play an important role in understanding the etiology and pathogenesis of preeclampsia.

LIMITATIONS AND RECOMMENDATIONS

The limitations of our study were the small sample size and median gestational age difference of preeclamptic pregnant females and normotensive pregnant controls. It is recommended that homocysteine and uric acid levels in early pregnancy and even before conception should be done to decrease the incidence and complications of preeclampsia. It is also recommended that supportive supplementations of folic acid, vitamin B_{12}, and B_6 can be tried to minimize the disorders related to amino acid metabolism, especially methionine, involved in the formation of homocysteine and uric acid as intermediates.

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Original Article

Spectrophotometric Analysis of Color Stability of Three Different Restorative Materials

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ABSTRACT

Objective: To compare in vitro the color stability of three different restorative materials by spectrophotometric analysis at time intervals of 24 hours, 1 week, 2 weeks, and 4 weeks after exposure to cola drink and tea.

Methodology: This in-vitro experimental study was conducted in the Applied Chemistry Research Center at PCSIR laboratory after ethical approval from Institutional Review Board (IRB) of FMH College of Dentistry Lahore. Three different restorative materials [(Biodentine™, Resin-Modified Glass Ionomer Cement (RMGIC), and Glass Ionomer Cement (GIC)] were evaluated for color stability. Eighteen disc-shaped specimens (20 × 2 mm) were prepared using an acrylic ring mould. Color coordinates ($L^*a^*b^*$, ΔL^*, Δa^*, Δb^*, and ΔE^*) were measured using a spectrophotometer. All the fabricated specimens were subdivided into two groups; A and B (n= 9 each) and immersed in distilled water for 24 hours for baseline color evaluation. Thereafter, the specimens of group A and B were immersed in two different beverages *i.e.* cola drink (n= 9) and tea (n= 9), respectively. The restorative materials were evaluated at intervals of 1 week, 2 weeks, and 4 weeks of storage. These specimens were subjected to pH cycling by immersion in demineralizing and remineralizing solution and immersed in cola drink and tea for 30 minutes for the entire duration of the study. Data was analyzed using Statistical Package for the Social Sciences (SPSS) version 20. The color changes (ΔE) were calculated and analyzed by Friedman's test to identify the significant group at a 5% confidence level.

Results: At 24 hours interval, the ΔE of all the restorative materials was statistically insignificant. The statistical analysis depicted that the ΔE of group B (tea) was significantly lesser than that observed with group A (cola drink) for Biodentine™ and RMGIC. On the contrary, ΔE of group A (cola drink) was observed to be lesser for GIC in comparison to group B (tea). The color change gradually decreased over the period of 4 weeks in both groups for all the restorative materials.

Conclusions: Mean color change after 4 weeks of immersion in cola drink and tea was most significant for Biodentine™ in comparison to RMGIC and GIC. However, RMGIC depicted better mean color stability than other restorative materials.

Keywords: *Color Stability. Spectrophotometer. Biodentine™. Glass Ionomer Cement (GIC). Resin-Modified Glass Ionomer Cement (RMGIC).*

INTRODUCTION

Replacement of a missing tooth or a part of the tooth structure has always been a challenging task for dental practitioners. Any effective permanent restorative material should be able to fulfill three main criteria *i.e.* optimal strength, dimensional stability, and ability to replicate natural esthetics for their clinical efficiency over a considerable period. Over the years, patient awareness and demand for esthetically appealing dental replacements have increased. Therefore, a multitude of restorative materials and treatment modalities have been developed to conform with the requirements of the aesthetic properties of restorative materials.[1]

A dental restorative material must not only mimic the natural dentition in color, translucency, and surface texture but should also exhibit long term resistance to color changes on exposure to a wide variety of discoloring substances.[2] Marginal or surface discoloration is one of the leading clinical shortfalls of tooth-colored restorations.[3] Tooth discolorations can generally be classified as extrinsic or intrinsic. Discoloration may also be due to physiochemical reactions in the deeper part of the body of the restorations, known as intrinsic discoloration.[1] Extrinsic discoloration is a broad term, comprising of adsorption and absorption.[3] Adsorption governs the external discoloration due to the accretion of dental plaque and surface stains, while the surface or subsurface color transitions, denoting superficial deterioration or slight ingress and reaction of staining agents with the superficial layer of restorative materials are due to absorption phenomenon.[1] Extrinsic discoloration is usually fostered by coloring agents in beverages and foods.[3] A variety of substances used routinely ultimately cause esthetic compromise. Many dietary factors have a prime role in the staining of oral tissues and dental restorations.[2] A multitude of studies exist on the beverage-dependent discoloration of tooth-colored restorations. The beverages used in most of these studies were coffee, tea, and wine, which are usually cognate with adult tooth stains.[4]

Conventional glass ionomer cement (GIC) are fluoride-releasing dental restorative materials that are prone to discoloration. Modern developments led to the production of newer materials such as composites, polyacid modified composites, and resin-modified glass ionomers (RMGIC); all of which have

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significantly better mechanical as well as esthetic properties. However, there is meager data available relating to the color stability of these hybrid restorative materials.[2]

Progressive tooth discoloration has also been reported to be the result of the penetration of endodontic materials into dentinal tubules.[5] Many endodontic procedures like; direct pulp capping, pulpotomy, perforation repair, and retrograde fillings require the placement of restorative materials in the coronal part of the tooth, which may cause tooth discoloration after some time of treatment completion.[5-6] Therefore, the color stability of endodontic materials is considered an important factor for clinical success; particularly in endodontically treated teeth.[7]

This mandates that the material selection should not rely entirely on biological and functional criteria alone, but aesthetic considerations should also be taken into account. [5,8]

Biodentine™ is a calcium silicate-based restorative material. This material is an advanced Mineral Trioxide Aggregate (MTA) based cement and therefore, it has been proposed as a "dentine replacement" material.[9] Earlier, MTA has been known for its excellent marginal adaptation, cell proliferation induction, and the formation of a high-quality hard tissue barrier. [10] Biodentine™ claims to have improved physical and handling properties, which were the shortfalls of MTA. This material has an array of clinical applications, including a variety of endodontic repairs and pulp capping.[11]

Discoloration can be assessed clinically to determine the outcomes over a long period of time but in-vitro analysis using a spectrophotometer are a prescribed standard for assessment of the quantity of color transition in restorative materials. It involves using systematized colorimetric equipment for matching and measuring color that provides accurate data about reflectance curve as a function of wavelength in the complete range.[1] This study was aimed to assess and compare the color stability of three different restorative materials, Biodentine™, RMGIC, and GIC, after exposure to frequently used beverages, cola drink and tea, which causes staining in restorations.

METHODOLOGY

This in-vitro experimental study was conducted at Applied Chemistry Research Center at PCSIR laboratory after ethical approval from IRB of FMH college of Dentistry Lahore. The total duration of the study was three months. A total of 54 specimens, 18 specimens for each restorative material i.e. Biodentine™, Glass Ionomer Cement (GIC), and Resin Modified Glass Ionomer Cement (RMGIC) were included in the study.

Specimen Fabrication:

An acrylic ring mould (20×2 mm) was used to fabricate standardized specimens of each restorative material**.** All specimens were made following the manufacturer's instructions for accurate material manipulation. Biodentine™ and RMGIC were manipulated in an amalgamator for 10 seconds to achieve a homogenous smooth mix. The mould was filled with the mixed materials individually and covered with a Mylar strip to ensure the surface smoothness and adaptation of the set materials. Glass Ionomer Cement was hand-mixed by following the standard protocols and the specimens were fabricated using a similar mould.

All the fabricated specimens of the three materials were subdivided into two groups A and B (n=9). Group A was cola drink (The Coca-Cola Company, Atlanta, GA) and group B was tea (one Lipton yellow label tea bag dipped in 200ml boiling water for 10 minutes). These specimens were preserved in distilled water for 24 hours at ambient temperature to prevent desiccation. Before the spectrophotometric analysis, the specimens were rinsed and then dried with blotting paper.

Baseline Color Evaluation:

The baseline color was measured with the help of a spectrophotometer (Nicolet Evolution Model 100 UV, Thermo Electronic Corporation) at 24 hours interval (T1). The color parameters were based upon an illuminating view geometry d/10 and average daylight (D65: 6504 K) standard. A white standard was used for calibration. The specimens were positioned on aperture individually, and values were recorded according to Commission Internationale de l'Eclairege L*a*b* color space (CIELAB).[12]

Specimen Processing For Staining:

After the baseline color evaluation, the specimens were immersed in two different beverages *i.e.* cola drink (group A) and tea (group B). The specimens were treated for pH cycling by bathing in the demineralizing solution for 7.5 hours and then its mineralizing solution for 16 hours daily. For this purpose, the demineralizing solution was prepared using 2.2mM $CaCl_2$, 2.2mM NaH_2PO_4, and 50mM Acetic acid of pH 4.8. The remineralizing solution was prepared using 1.5mM $CaCl_2$, 0.9mM NaH_2PO_4, and 0.15 M KCl adjusted to pH 7. These solutions acted as a preservation medium for the specimens to simulate the ongoing changes in the oral environment.

The specimens were daily withdrawn from pH cycling solutions and bathed in cola drink and tea for 30 minutes for the entire duration of the study. After immersion and pH cycling, the color change was evaluated after 1 week (T2), 2 weeks (T3), and 4 weeks (T4).

Measuring the Color Change:

All the readings were measured at specified time intervals of 1 week (T2), 2 weeks (T3) and, 4 weeks

(T4). The values of ΔL* (degree of lightness), Δa* (hue i.e. color), and Δb*(Chroma i.e. vividness/dullness) were calculated after taking the three individual measurements on the spectrophotometer (Nicolet Evolution Model 100 UV, Thermo Electron Corporation).[13] Resistance to discoloration was expressed in ΔE unit and estimated from the mean ΔL*, Δa*, and Δb* values for each specimen by using the formula $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$.[14]

STATISTICAL ANALYSIS

The study data gathered was entered and processed with Statistical Package for the Social Sciences (SPSS) version 20.0. Descriptive statistics i.e. mean and the standard deviation were calculated. The comparative assessment of mean color change (ΔE) as a function of two different beverages on specified time intervals was obtained using the Friedman test and the significant groups were identified at a 5% confidence level. Wilcoxon signed-rank test was employed to assess the mean color change between the two groups (i.e. A and B).

RESULTS

Mean color change (*ΔE*) of the specimens bathed in distilled water for the first 24 hours (T1), followed by immersion in cola drink at different time intervals (*i.e.*T2, T3, and T4) is shown in Table 1. The mean color change (*ΔE*) of the specimen immersed in distilled water for the first 24 hours (T1) and then in tea at different time intervals (*i.e.*T2, T3, and T4) is depicted in Table 2. The overall effects of both the beverages on Biodentine™, GIC, and RMGIC are illustrated in Figures 1, 2, and 3, respectively.

Closer to zero reading of *ΔE* is suggestive of increased staining (*i.e.* color change) on spectrophotometric analysis. The statistical interpretation depicted that the *ΔE* of group B (tea) was significantly lesser than that of group A (cola drink) for Biodentine™ and RMGIC (Figure 1 and 3). On the contrary, *ΔE* of group A (cola drink) was observed to be lesser for GIC in comparison to group B (tea) (Figure 2). The most significant mean color change was recorded for Biodentine™ in both groups A and B from T1 to T4 intervals (Table 1 and 2). The color change depicted a gradual increasing trend over the period of 4 weeks (T4) in both the groups for all the restorative materials (Table 1 and 2). The only nearly stable observations were recorded for RMGIC at T2 (Mean ±SD =71.65±1.00) and T3 intervals (Mean ±SD =71.63±1.14) in group B (Table 2).

The overall results of the study revealed that the maximum mean color change appeared to be at T4 of immersion in cola drink and tea for all the restorative materials. The mean color change in all the restorative materials gradually increased over a period of 4 weeks. The most significant increase in mean color change was observed for Biodentine™ as compared to GIC and RMGIC (Table 1 and 2). It is thus hypothesized, based on the statistical interpretation of the data, that RMGIC relatively indicated better mean color stability than that manifested by Biodentine™ and GIC (RMGIC; p=0.016 and p=0.092 for group A and B, respectively) (Table 1 and 2).

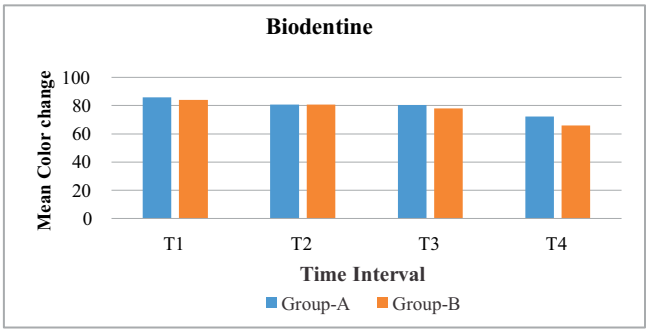

Figure 1: Mean Color Change (ΔE) of Biodentine™ After Storage in Cola Drink (Group A) & Tea (Group B)

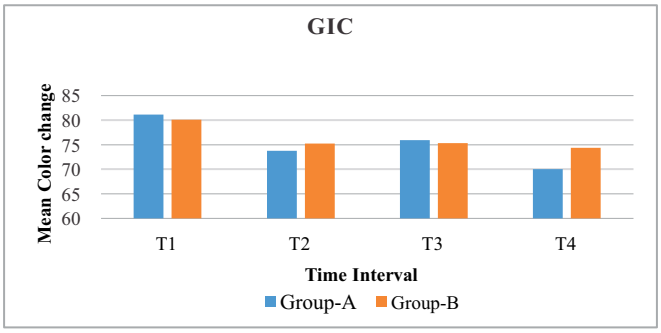

Figure 2: Mean Color Change (ΔE) of GIC After Storage in Cola Drink (Group A) & Tea (Group B)

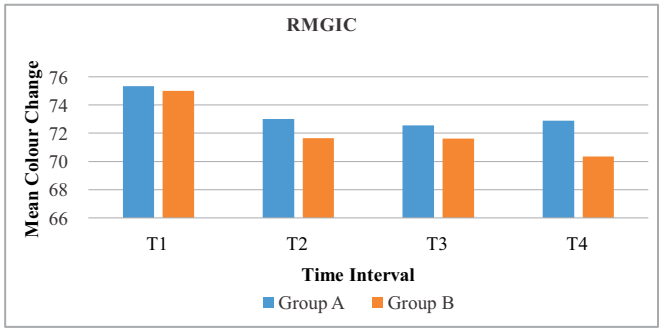

Figure 3: Mean Color Change (ΔE) After Storage in Cola Drink (Group A) & Tea (Group B) on RMGIC

Table 1: Mean Color Change (ΔE) After Storage in Cola Drink (Group A) on Three Different Restorative Materials at Specified Time Intervals (n=9)

Material	Time Intervals	Mean±SD	p-value
Biodentine™	T1	85.83±0.64	0.000
	T2	80.76±1.99	
	T3	80.34±1.77	
	T4	72.17±1.96	
GIC	T1	81.18±2.89	0.000
	T2	73.77±2.07	
	T3	75.93±1.62	
	T4	70.04±0.74	
RMGIC	T1	75.34±1.10	0.016
	T2	73.01±2.28	
	T3	72.56±1.73	
	T4	72.88±1.56	

T1: 24 hours (Baseline), T2: 1 week after immersion, T3: 2 weeks after immersion, T4: 4 weeks after immersion

Table 2: Mean Color Change (ΔE) After Storage in Tea (Group B) on Three Different Restorative Materials at Specified Time Intervals (n=9)

Material	Time Intervals	Mean±SD	p-value
Biodentine™	T1	84.04±2.72	0.000
	T2	80.57±1.26	
	T3	78.01±2.49	
	T4	65.80±2.96	
GIC	T1	80.11±0.82	0.000
	T2	75.21±1.39	
	T3	75.33±1.10	
	T4	74.38±0.58	
RMGIC	T1	74.99±2.18	0.092
	T2	71.65±1.00	
	T3	71.63±1.14	
	T4	70.35±1.06	

T1: 24 hours (Baseline), T2: 1 week after immersion, T3: 2 weeks after immersion, T4: 4 weeks after immersion

DISCUSSION

The color stability of three different dentine-replacement restorative materials namely Biodentine, glass ionomer cement (GIC), and resin-modified glass ionomer cement (RMGIC) was evaluated and compared by spectrophotometric analysis after immersion in tea and cola. A standardized solution of tea (one Lipton yellow label tea bag dipped in 200ml boiling water for 10 mins) and cola drink (The Coca-Cola Company, Atlanta, GA) were used for immersion of specimens to replicate the oral environment, where these beverages may be consumed occasionally or regularly.

The surface discoloration is closely related to the thickness of the restoration, Therefore, the thickness of experimental specimens used in this study was 2 mm as per International Orgainzation for Standardization standards [12] and spectrophotometry requirements.[5] This

thickness of specimens has been reported as technically acceptable to reduce the background effect and minimize error.[14-16] The American Dental Association (ADA) suggests the application of the CIELAB color differential system for estimating chromatic differences and changes which was employed for this study.[17]

In this study, a pH cycling procedure was employed to imitate oral environmental conditions. It has previously been demonstrated that fluoride-releasing materials have a greater ion release potential when exposed to pH variations than when solely kept in artificial saliva or saline.[2] These facts could instigate lower color stability of GIC in comparison to RMGIC as evident from the results of the present study and studies conducted by Bezgin et al., and Savas et al.[18,19] They concluded that there was a significant ($p<0.05$) change in the level of staining of GIC after immersion in cola drink for 28 days.

Resin-Modified Glass Ionomer Cement (RMGIC), being resin-based possesses comparatively low fluoride release, and for this reason, it has shown the least amount of discoloration due to the reduced ionic interchange between the material and the environmental solutions (Table 1 and 2) (Figure 2 and 3). Furthermore, the susceptibility of resin-based materials, even if it is to a lesser extent, may be accredited to their extent of water sorption and hydrophilic characteristics of the resin matrix.[18,19] This denotes if the resin component can absorb water, it is also likely to absorb other staining fluids like tea and coffee.[5]

In this study, the maximum mean color change took place after 4 weeks (T4) of storage in cola drink and tea solutions for all three restorative materials. Our findings are in contradiction to the observations of a previously conducted study. Hotwani et al., delineated in their work that staining after 1 week of immersion differed remarkably from all the following weeks and the maximum amount of color change appeared during the first week.[20] This difference in observations can be ascribed to the choice of test restorative materials as Biodentine™ was not introduced at that point in time and this material has the greatest influence on the results of the present study.

The highest mean color change was observed for Biodentine™ when compared to GIC and RMGIC of immersion in both the beverages (Table 1 and 2). These findings may be attributable to the fact that Biodentine™ possesses entirely different physicochemical properties than GIC and RMGIC.

Calcium silicate-based materials are hydraulic, self-setting materials with intrinsic physiochemical properties appropriate for pulp therapy. Biodentine™ exhibits superior clinical physical properties such as good sealing, increased compressive strength, decreased porosity, higher density, bioactivity, the release of ions acting as epigenetic signals, the immediate formation of calcium hydroxide, biomineralization ability, biointeractivity, and color stability compared to mineral trioxide aggregate (MTA).[21] Biodentine™ with Chlorohexidine (CHX) should be avoided because of its propensity to cause clinically perceptible severe discoloration.[5] In contradiction to this, a number of recent studies have depicted high color stability of Biodentine™.[22,23] In the present study, Biodentine™ demonstrated the maximum amount of discoloration (Figure 1). This may be ascribed to the coarse surface texture and hydrophilicity of the set material.

In the present study, group B (tea) demonstrated a more pronounced mean color change in comparison to group A (cola drink) except for T2 and T4 in GIC (Figure 1, 2, and 3). This finding may be attributable to the concentration of the tea solution used in this study.

CONCLUSIONS

It can be concluded that the most significant increase in mean color change has been observed for Biodentine™ when compared to GIC and RMGIC. It can be enunciated that RMGIC manifests greater resistance to staining in comparison to the Biodentine™ and GIC at each time interval.

LIMITATIONS AND RECOMMENDATIONS

The limitations of the current study are that the impact of only two beverages (cola drink and tea) on the color change of three different restorative materials was evaluated. Also, only a single thickness of the test specimens was used (i.e. 2mm). Samples used in this study had smooth surfaces for standardization requirements. However, in clinical situations, the majority of cement does not possess a flat and absolute finished surface and the pH values of the oral cavity vary amongst individuals.

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Original Article

Microalbuminuria in Non-Diabetic and Non-Hypertensive Patients with Acute Myocardial Infarction

Muhammad Suleman Khan, Muhammad Saeed Afridi, Muhammad Ammar, Matti-ur-Rehman

ABSTRACT

Objective: To determine the level and frequency of microalbuminuria in non-diabetic and non-hypertensive patients with acute myocardial infarction (AMI).

Methodology: It was a cross-sectional comparative study that was conducted at the Emergency department of Punjab Institute of Cardiology, Lahore. The study was approved by the hospital ethical committee. Non-probability purposive sampling technique was used. A total of 200 subjects were included in this study which were divided into groups A & B. Group A included normotensive and non-diabetic diagnosed patients of acute myocardial infarction (AMI) of both genders aged between 30-70 years. Group B comprised of age-matched normal healthy controls. Spot urine samples of study participants of both groups were sent to the Pathology department of Punjab Institute of Cardiology for the assessment of microalbuminuria.

Results: Group A consisted of 53 males and 47 females. The mean age of the patients in group A was 50.21±8.92 years and 51.49±6.17 years in group B. In group B there were 41 males and 59 females. The mean urinary microalbumin level in group A was 91.14±63.73mg/24 hours and group B was 10.11±5.01mg/24 hours. Microalbuminuria in group A was statistically significant (p-value=0.03) as compared with the controls.

Conclusion: Non-diabetic and non-Hypertensive diagnosed patients of AMI had a statistically significant frequency of microalbuminuria. Thus, microalbuminuria can be considered as a risk factor for ischemic heart disease and should be checked routinely for screening of cardiovascular diseases.

Keywords: *Microalbuminuria. Non-Diabetic. Non-Hypertensive. Myocardial Infarction.*

INTRODUCTION

Acute myocardial infarction (AMI) is the most prevalent cardiovascular disease in the developed countries and its mortality rate is high. The common risk factors for cardiovascular diseases are hypertension, hyperlipidemia, atherosclerosis, and smoking.[1] The association of microalbuminuria with cardiovascular diseases, hypertension, and dyslipidemia is known. Urine microalbumin is a recognized marker of cardiovascular diseases and nephropathy in hypertensive and diabetic patients. The duration of diabetes mellitus and the level of micro or macroalbuminuria determine the risk for the development of atherosclerosis.[2] Microalbuminuria is also associated with increased risk of cerebrovascular and cardiovascular morbidity and mortality in non-diabetic patients.

Microalbuminuria is defined as persistent urinary excretion of ≥30mg/24 hours of albumin per day.[3] Individuals with microalbuminuria have severe subclinical atherosclerosis.[4] In non-diabetic hypertensive individuals with cardiovascular problems microalbuminuria was found in about 60%.[5] Albuminuria has been associated with dyslipidemia, obesity, impaired glucose tolerance, insulin resistance, and essential hypertension. Patients with albuminuria have high left ventricular mass and an increased risk of MI. It leads to generalized vascular endothelial dysfunction. It increases of risk of heart failure, stroke, MI, and cardiovascular deaths.[6]

In diabetic individuals, microalbuminuria is an established cause of endothelial dysfunction. In non-diabetics, it is likely to be a sign of malfunctioning of the endothelium of the kidneys. In hypertensive patients, von-Willebrand factor levels are increased with microalbuminuria, which is highly related to vascular thrombosis and can cause an increase in end-organ damage or even failure, cardiovascular, and cerebrovascular morbidity and mortality. It is also associated with cardiovascular morbidity in non-hypertensive patients.[7]

Microalbuminuria can be considered an early and strong predictor of high risk of myocardial infarction in the absence of other risk factors.[8] The study was carried out to evaluate the significance of microalbuminuria as a useful marker in patients of acute myocardial infarction without diabetes mellitus and hypertension. This would help to prevent the complications of ischemic heart disease.

METHODOLOGY

It was a cross-sectional comparative study which was conducted at the Emergency department of Punjab Institute of Cardiology, Lahore. The study was approved by the hospital ethical committee. Non-probability purposive sampling technique was used. Two hundred subjects were included in this study which were divided into groups A & B. Informed consent was taken from all the study participants.

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Group A included normotensive and non-diabetic diagnosed patients of acute myocardial infarction of both genders aged between 30-70 years. Group B comprised of age-matched normal healthy controls. Patients with known nephropathy and serum creatinine higher than 1.5mg/dl, history of diabetes, hypertension & systemic infection were excluded from the study. Diagnosis of AMI was based on electrocardiogram (ECG) findings and levels of cardiac markers. Blood pressure was measured and glycosylated haemoglobin (HbA1C) were measured in both group A and B. Normal mean range of mean HbA1C is between 4-5.6%. Spot urine samples of the study participants of both groups were sent to the Pathology department of Punjab Institute of Cardiology for the assessment of microalbuminuria. The normal urinary microalbumin level is <30mg/24 hours.

STATISTICAL ANALYSIS

The data was analyzed using Statistical Packages for the Social Sciences (SPSS) version 23.0. Qualitative variables like microalbuminuria were calculated as percentages and frequencies. Quantitative variables like age were calculated as mean and standard deviation. An independent t-test was used for comparison of both groups. A p-value ≤0.05 was considered as a cut-off for significance.

RESULTS

Group A consisted of 53 males and 47 females. The mean age of the patients in group A was 50.21±8.92 years and 51.49±6.17 years in group B. In group B there were 41 males and 59 females.

In group A, 50.33% had ST-elevation myocardial infarction (STEMI) & 49.67% patients had non-ST-elevation myocardial infarction (NSTEMI). Mean HbA1C in group A and B were 5.1±2.0% and 4.9±3.1% respectively. The mean blood pressure of the patients in group A was 118/75mmHg and 120/81mmHg in group B.

Comparison of the HbA1C level between group A and B was statistically non-significant (p-value=0.8). The mean blood pressure of the study subjects in group A was also found statistically non-significant (p-value=0.9) as compared to the control group B (Table 1).

The mean urinary microalbumin level in group A was 91.14±63.73mg/24 hours and 10.11±5.01mg/24 hours in group B (Figure 1). This increased excretion of microalbumin in group A was statistically significant (p-value=0.03) when compared with controls.

The mean urinary albumin concentration of the patients with NSTEMI was 79.63±13.14mg/24 hours and in patients with STEMI was 81.55±14.94 mg/24 hours. The comparison of microalbuminuria in patients with STEMI and NSTEMI was found statistically non-significant (p-value=0.84)

Table 1: Study Variables of the Subjects Included in Group A and B.

Study Variables	Group A	Group B	p-value
Mean HbA1C Levels (%)	5.1±2.0	4.9±3.1	0.8
Mean Blood Pressure (mmHg)	118/75	120/81	0.9
Mean Urinary Microalbumin (mg/24 hours)	91.14±63.73	10.11±5.01	0.03*

*Significant p-value

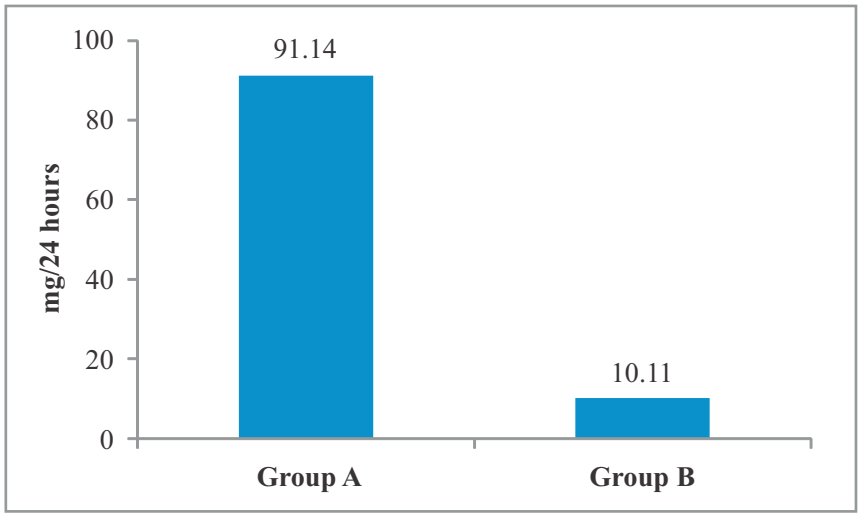

Figure 1: Urinary Microalbumin Levels (mg/24 hours) in Study Groups

DISCUSSION

Microalbuminuria is related to cardiovascular morbidity and is a risk factor for cardiovascular diseases. Our results showed significantly high (p-value=0.03) microalbuminuria in non-diabetic, normotensive patients with acute myocardial infarction as compared to control. Similar results were found in another study which showed that the microalbuminuria was associated with AMI without the presence of other risk factors like hypertension and diabetes.[9]

Mok and his colleagues also found that albuminuria is a strong predictor of subsequent outcome in patients with acute myocardial infarction, which suggests that attention must be given to albuminuria in addition to renal function in high risk patients.[2] A study was conducted in India to determine the association of microalbuminuria with myocardial infarction. They included 50 non-diabetic & non-hypertensive patients of acute myocardial infarction and 50 healthy age-matched controls. They found increased micro-albuminuria in non-diabetic, normotensive patients of acute myocardial infarction as compared to the controls (p-value<0.001).[10]

Åkerblom et al. reported that microalbuminuria and non-ST-segment elevation acute myocardial infarction have elevated morbidity and mortality which is independent of estimated glomerular filtration rate.[3] Another study revealed that moderate micro-albuminuria and a rise in serum creatinine were considered as increased risk factors for cardiovascular disease.[6]

Our results showed that the mean urinary albumin concentration of patients with STEMI & NSTEMI was 79.55±14.94mg/24 hours and 81.63±13.14mg/24 hours respectively. Comparison of microalbuminuria in patients with STEMI and NSTEMI was statistically non-significant (p-value=0.84). On the contrary, Al-Saffar et al. found a strong correlation of micro-albuminuria in NSTEMI patients.[11]

Our results indicated that microalbuminuria is related to acute myocardial infarction without the presence of other risk factors like diabetes mellitus, hypertension, and renal disease. A study conducted by Basu et al., included 50 diagnosed patients of acute myocardial infarction and 50 healthy controls who were sex & age-matched. The patients with diabetes mellitus and hypertension were excluded. They found increased microalbuminuria in non-hypertensive and non-diabetic patients with myocardial infarction.[12] A study conducted in Mexico, also reported similar results.[13] These results showed that microalbuminuria in these patients was not present due to kidney disease or other risk factors like diabetes and hypertension. Thus microalbuminuria may cause endothelial damage and systemic inflammatory response in patients with MI.[14,15]

CONCLUSION

Non-diabetic and normotensive diagnosed patients of AMI had a statistically significant frequency of microalbuminuria.

LIMITATIONS AND RECOMMENDATIONS

It is suggested to conduct further multi-centered studies with a larger sample size to further investigate the pathophysiology of microalbuminuria in cardio-vascular diseases.

Microalbuminuria can be considered as a risk factor for ischemic heart disease and should be checked routinely for screening of cardiovascular diseases.

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Original Article

Students' Perception of the Mentorship Program at a Private Medical College of Lahore

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ABSTRACT

Objective: To analyze the perception of 1st Year MBBS students regarding the mentorship program in Shalamar Medical & Dental College.

Methodology: It was a descriptive cross-sectional study that was conducted at Shalamar Medical and Dental College, Lahore. A questionnaire containing 28 items was distributed among all students on their return from the 1st professional exam. The questionnaire consisted of open and closed-ended questions where the latter were based on the Likert scale.

Results: A total of 110 students participated in the study, out of which 69 were females, 41 males. Among the positive responses, 72% of the participants agreed that mentors were readily available for group sessions and 59% agreed to mentors' availability for individual sessions. Regarding the role of mentors in encouraging students' involvement in campus committees, time management, and helping them unite their personal and academic life; the students' response rate was 76%, 58%, and 56% respectively. The most commonly discussed issues were time management and confidence building. Nearly half of respondents received encouragement to conduct research, help in improving presentation skills, and increased knowledge about ethical dilemmas. Regarding constructive feedback, 68% of the students found their mentors beneficial for themselves, and 49% considered mentors as role models. Overall, 64% of students felt that the program had benefitted them in some way.

Conclusion: The students had a positive perception of the mentorship program and 64% of the students believed that they benefitted from it. The students felt that the constructive feedback provided by mentors was helpful and it helped them not only in gaining confidence but also in managing time well. It was concluded that to improve the program, the frequency and duration of meetings should increase.

Keywords: *Mentorship. Behaviour. Counseling. Mentor. Mentee. Perception.*

INTRODUCTION

Mentorship programs are initiated in medical institutes to facilitate the students' academic and personal development.[1] It is a process whereby a senior faculty (mentor) provides guidance, counseling, friendship, and support to juniors (mentees) to assist them in achieving their career goals.[2] Medical students are expected to be at the top in their academic performance to connecting with patients and competing for better professional opportunities. Overall, the experience is taxing. Burnout in medical students ranged from 27% to 75%, worldwide, and suggested mentorship programs as one of the ways to reduce burnout in students.[3] A similar study was carried out in Pakistan and it was found that nearly half (47%) of students experienced burnout at some point in their undergrad.[4] Additionally, it is believed that mentoring produces successful doctors with a greater degree of career satisfaction as it helps the medical students to streamline their goals and work towards them.[5,6] However, the success of the mentorship program lies in the goal of the mentor-mentee relationship, the framework of the program, and the characteristics of mentors and mentees.[7] A successful mentorship program helps mentees garner academic, social, and mental benefits from the relationship. It decreases their stress level which in turn improves their emotional well-being.[8] Literature search reveals that mentees receive guidance related to career or prospective research areas from their mentors. This is why a major role of mentors is described as being a good role model.[9]

A mentorship program that involves mentees to formally meet their mentors has shown to improve students' perception about faculty as compared to the informal mentor-mentee relationship that develops in the course of study. The students feel that their teachers are more invested in their progress.[10] Mentorship programs pave the way for improved communication between mentors and mentees which helps them overcome many issues such as inferiority complex.[11] The success of a mentorship program depends on the relationship between mentors and mentees which in turn affects the satisfaction of mentees with the program.

In Pakistan, only a few Medical colleges have initiated a structured mentorship program. Dow International Dental College Karachi, Rawalpindi Medical University, Bahria University, University College of Medicine and Dentistry, UOL, and Peshawar Medical College are a few medical institutions that have started a structured mentorship program. Shalamar Medical and Dental College (SMDC) also initiated a mentorship program in 2014 for 1st year MBBS. The program includes 12 senior faculty members who are each allocated 12 students randomly. The group meets with their mentors formally and every individual student is welcome to contact their mentor informally

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whenever they feel the need. For the smooth running of the program and to see whether the program is achieving its goal, it is necessary to review it. It was noted that the evidence of an effective structured mentorship program and students' perception about it was deficient in Pakistan.

The rationale of the current study was to explore the perception of 1st year MBBS students regarding the mentorship program, so a survey was conducted to analyze the satisfaction of students with their mentors and the program. The study aimed to assess students' views on the role played by their respective mentors in their professional and personal growth. Based on the suggestions; the shortcomings were identified and worked upon.

METHODOLOGY

This study dealt with perceptions and suggestions of 1st year MBBS students at SMDC regarding the mentorship program as they are the major stakeholders. After obtaining ethical permission from the Institutional Review Board of Shalamar Medical and Dental College, the study was carried out to analyze the perception of the students regarding the mentorship program and to utilize the feedback to improve the program. It was a descriptive cross-sectional study that was conducted at Shalamar Medical and Dental College. A total of 7 group sessions were organized from December 2018 to September 2019 for 1st year MBBS students. The questionnaire was disseminated among all the students of 1st year MBBS enrolled in 2018. All the students of 1st year MBBS enrolled in 2018, were included in the study, and the students who never attended a mentor-mentee session and never met with their respective mentors (during or after mentorship sessions) were excluded from the study. The consent form was attached to the questionnaire when it was disseminated to the students. After signing the consent form, it was detached from the research questionnaire to ensure confidentiality. The information rendered by the participants was kept strictly confidential. The feedback forms were marked anonymously.

This study utilized questions taken from a questionnaire used in a similar study previously.[8] This questionnaire contained 24 items and was distributed among all the male and female students (age range: 18-25 years) as soon as they returned from the 1st professional exam. The questionnaire consisted of open and closed-ended questions. The first part of the questionnaire had questions that the students responded to by choosing a scale from 1-5 which ranged from "strongly disagree" to "strongly agree". The second part dealt with the questions that were responded to by students again on the scale of 1-5 with 1: not at all helpful, 2: somewhat helpful, 3: helpful, 4: very helpful, and 5: not applicable. The open-ended questions asked the respondents to recall the positive aspects of the program and give suggestions to improve the aspects that they are dissatisfied with. The form did not contain any identifiable information about the participating students.

STATISTICAL ANALYSIS

The data was analyzed by Statistical Package for the Social Sciences (SPSS) version 20 using frequencies and an independent t-test. A p-value ≤ 0.05 was considered significant. For the open-ended questions, the suggestions given by the respondents were read individually by all authors. After a discussion between all the authors, the recurring suggestions were identified and comments were grouped under categories. The comments that did not come under the defined category were included in the "others" category.

RESULTS

A total of 110 students participated in the study by filling the questionnaire. Out of 110 participants, 69(63%) were females and 41(37%) were males. All the participants, except one, had taken at least one group or individual session with the mentors. The participants who had not taken any session with the mentor were excluded from the study. Out of the total participants, 58% had taken one or more individual sessions with their mentors and 52% of the respondents had taken more than 3 scheduled group sessions (Table 1). Detailed analyses of all the questions are given in Table 2. Among the positive responses, 72% of the participants agreed that their mentor was readily

Table 1: Number of Group Sessions Attended by Respondents

Respondents	Number of group sessions taken by respondents		
	≤3 sessions	>3 sessions	Total
Male	27	14	41
Female	31	38	69
Total	58	52	110

available during sessions (Item 2, Table 2) and 59% of the participants agreed that their mentors were approachable for individual sessions when the need arose (Item 5, Table 2). Similarly, 56% of the students had stated that they discussed the issue of time management with their mentors and received help (Item 14, Table 2). A significant majority of the students (76%) expressed discontent over their mentors for not encouraging their involvement in committees on campus (Item 3, Table 2). According to 54% of students, their mentors did not understand that they had a life outside campus so did not help them in uniting the two (Item 4, Table 2). Overall, 64% of students felt that the program had benefitted them in one way or another (Figure 1). When the data was stratified based on gender, no significant difference was seen in responses obtained from males and females (p-value=0.83).

Table 2: Responses of Study Subjects

Sr. No.	Questions	Strongly agree	Agree	Neutral	Disagree	Strongly disagree
1	I have benefitted from the mentoring relationship.	21%	30%	30%	5%	14%
2	My mentor is readily available	26%	46%	20%	8%	0%
3	My mentor has involved me in committees within the institute.	2%	17%	5%	30%	46%
4	My mentor understands that I have a life outside of the institute and helps me to integrate my responsibilities.	10%	14%	22%	41%	13%
5	Whenever individual attention is required, my mentor is available & approachable.	22%	37%	31%	6%	4%
		Not at all helpful	**Somewhat Helpful**	**helpful**	**Very helpful**	**Not applicable**
6	Helping me determine my career goals	35%	33%	12%	20%	0
7	Encouraging development of my own research ideas	30%	10%	25%	15%	20%
8	Providing me with constructive feedback	9%	49%	10%	9%	23%
9	Developing my leadership skills	35%	20%	19%	20%	6%
10	Identifying a balance between my career and personal goals	26%	41%	18%	5%	10%
11	Providing emotional support around academic-related issues	25%	10%	15%	35%	15%
12	Providing emotional support around personal issues	34%	10%	37%	13%	6%
13	Being a role model for me	43%	20%	23%	6%	8%
14	Helping me in managing time effectively	30%	25%	23%	8%	14%
15	Facilitating me in conflict resolution when needed	13%	37%	29%	15%	6%
16	Guiding me with career counseling	30%	1%	17%	41%	11%
17	Improving my Presentation skills	43%	15%	15%	18%	9%
18	Helping me in clarifying Ethical issues	36%	28%	16%	5%	15%

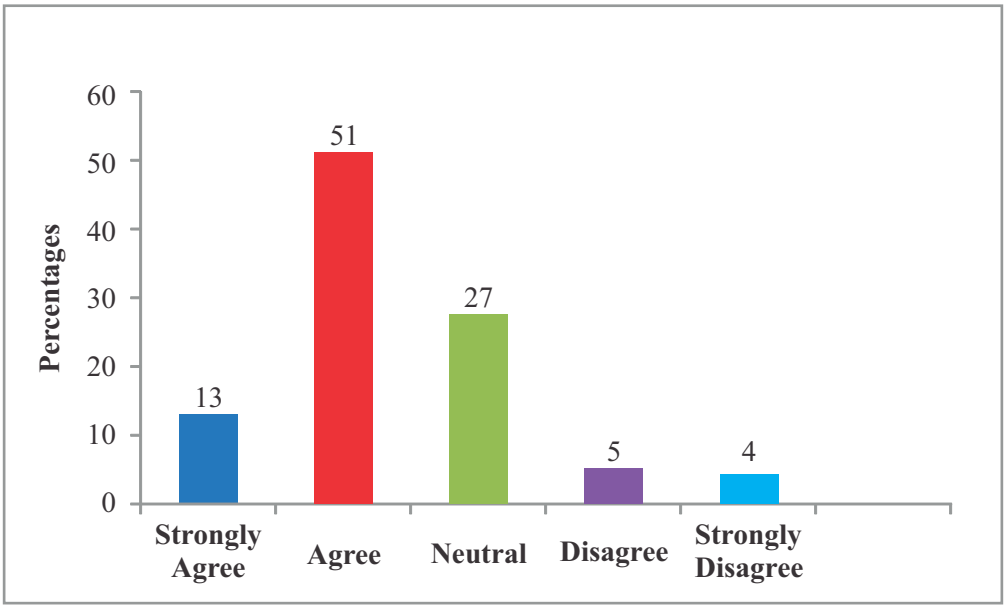

Figure 1: Percentage of Students who Benefitted from the Mentorship Program

Regarding emotional support related to personal and academic issues, 60% of the students felt that their mentors helped them cope with them (Items 11 & 12, Table 2). Half of the students admitted that their mentors encouraged them to conduct research (Item 7, Table 2). Out of these 5% had discussed research specifically with their mentors during sessions. Career counseling by mentors was considered helpful by 59% of the respondents and they agreed that their mentors guided them to identify between career and personal goals (Item 16, Table 2). Almost 59% of the students found their mentors to be helpful in developing leadership (Figure 2, Item 9, and Table 2).

Nearly half of the respondents received help in improving presentation skills (48%) and increasing their knowledge about ethical dilemmas (49%) (Items 17 & 18, Table 2). Regarding constructive feedback, 68% of the students found their mentors beneficial for themselves (Item 8, Table 2). The mentors were considered role models by 49% of the students, while 43% disagreed and the rest were indecisive. Different issues discussed by students with their mentors are shown in Figure 3.

The responses to open-ended questions were divided into various categories to ease the data analysis. Different responses obtained about the best aspects of the mentorship program are shown in Figure 4. Similarly, the students were asked about any suggestions to improve the mentorship program. Most suggestions involved increasing the time allotted to the sessions as well as the number of individual sessions. Students were dissatisfied with the unavailability of mentors due to other commitments (Figure 5).

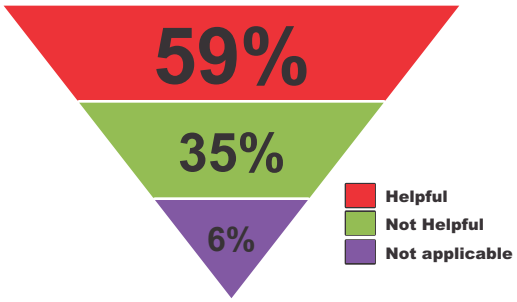

Figure 2: Percentage of Respondents who Felt their Mentors Helped in Developing Leadership Skills

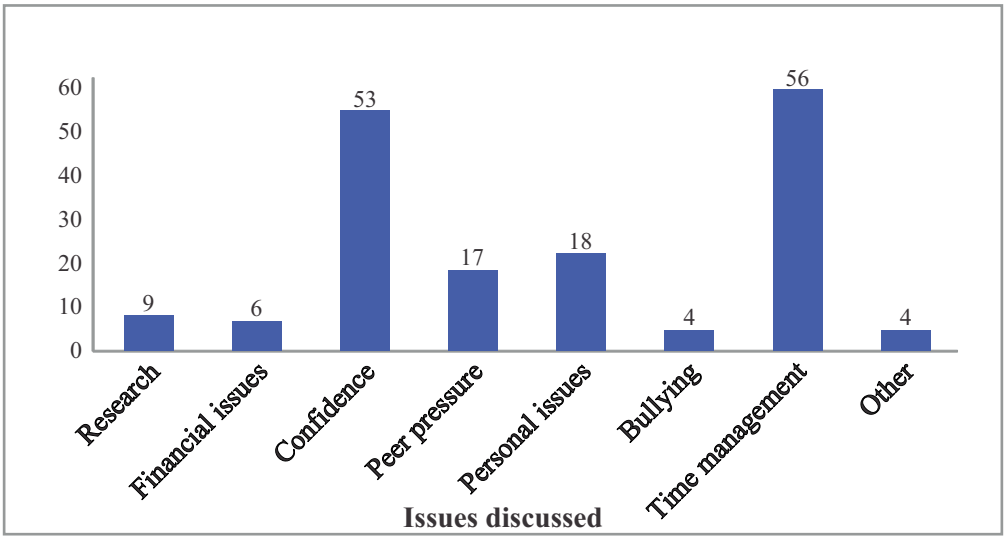

Figure 3: Frequency of Different Issues Discussed with Mentors

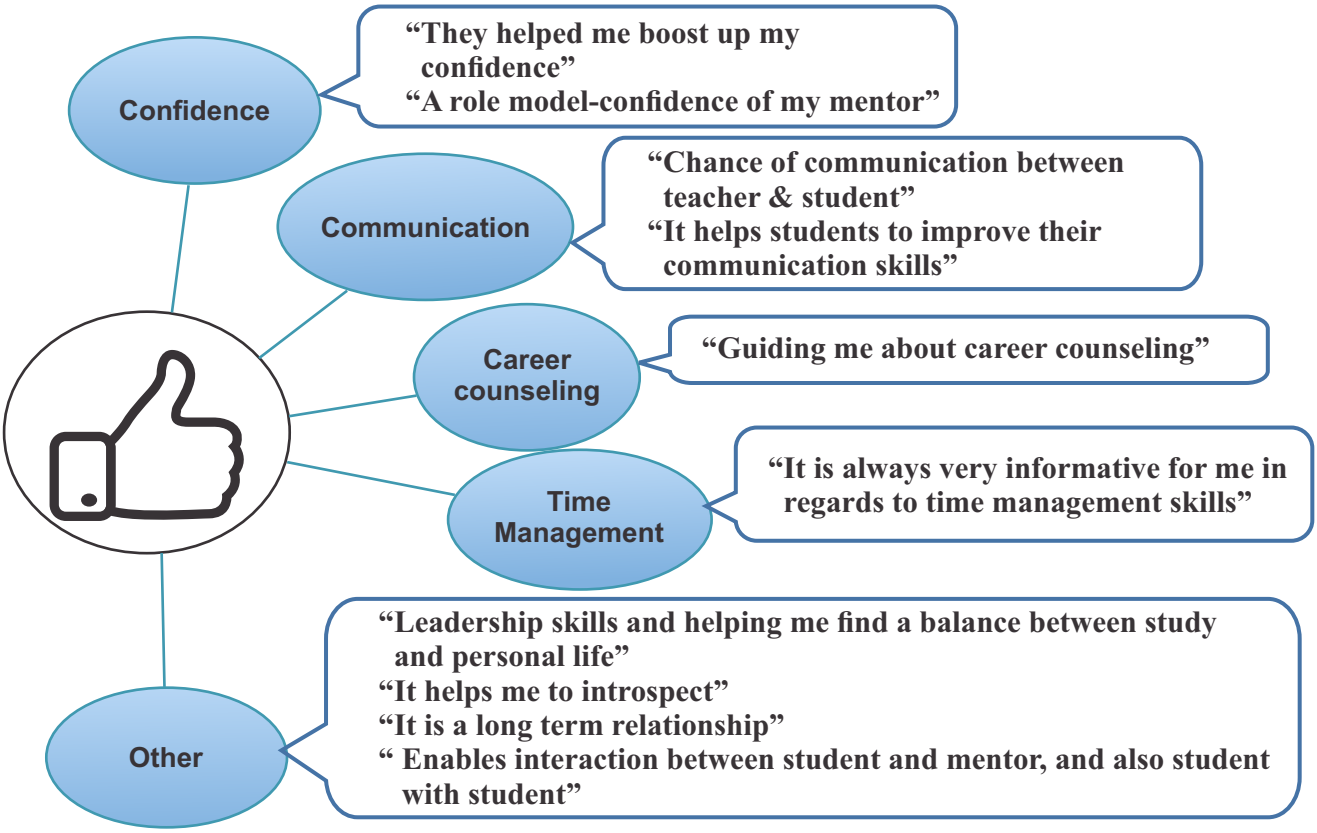

Figure 4: Different Responses by Students for the Best Aspects of the Mentorship Program

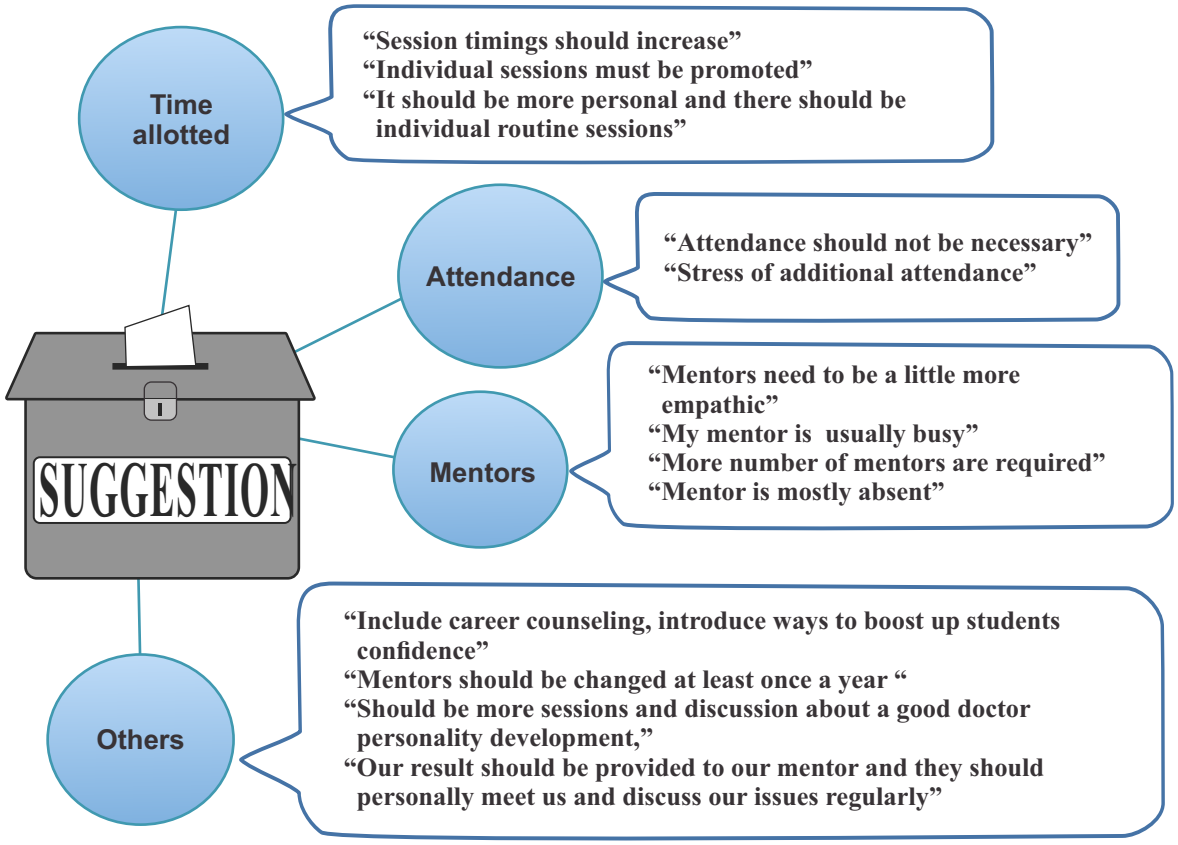

Figure 5: Different Suggestions Given by Students to Improve the Mentorship Program

DISCUSSION

This study helped in evaluating the effectiveness of the mentorship program. Our results depict an overall satisfaction of students (64%) with the program which is similar to the results obtained by Sparshadeep et al.[11] In our study, the mentors were rated highly by 68% of the students for providing constructive feedback. The ability to provide helpful feedback was highlighted previously as well.[12] Another study showed that a mentorship program helps students to improve academically and reduce anxiety among 1st year MBBS students.[13] Apart from effective feedback, our study identified several strengths related to the relationship between mentors and mentees, which included increased self-confidence of mentees and the development of better communication skills. Effective mentoring has positive effects on the mentee's communication skills which results in the development of positive interpersonal and communication skills.[14] Literature has identified certain pre-requisites that an individual must possess to become an effective mentor for the students. These include their motivation towards research and teaching goals, academic competencies as well as their high-quality communication skills, and ability to provide emotional support.[15] Our study revealed, that these positive attributes of mentors have resulted in mentees considering their mentors as role models for themselves. Mentors have a huge responsibility to act as role models with a positive personality and professional traits for their mentees.[16] Therefore, the success of any mentoring program relies on the ability of mentors to be available and encouraging for their mentees. Mentors exhibit positive traits and assist in widening the professional circle of mentees by incorporating leadership and ethical values. Additionally, they should also work on the personal development of the mentees.[9]

Five percent of mentees in the current study mentioned that they received guidance regarding research from their mentors. Comparatively, a larger proportion (38%) of respondents said that they received guidance about research from mentors in a study carried out in the USA.[6] The difference is due to the professional level of mentees in both studies. Our study was carried out with 1st year medical students unlike postgraduate students in the aforementioned study. In medical colleges under the University of Health Sciences, the research component is not compulsory in 1st year of

MBBS which might explain the reason for the difference in responses. Students might not feel the need to discuss research with the mentors.[17] Two-third of the mentees agreed that their mentors were readily available to discuss their issues, however, the remaining mentees highlighted that their mentors were unavailable for sessions. One of the reasons for their absence as identified by students was their busy schedule. After analyzing the record of mentors' attendance in the sessions, we gathered that 25% of the mentors had attended less than half of the sessions. This is in accordance with the results obtained from the students as nearly 30% of the students disagreed when asked if their mentors were readily available. The students also suggested that the number of meetings should increase and many students believe that individual sessions should be encouraged. The increase in the frequency of the mentor-mentee sessions has been demanded by mentees in literature as well in which the 1st year medical undergraduates met their mentors twice a month.[11] The students felt hesitant discussing certain issues in group meetings. This could be the reason why our program rated lower when students were asked whether their mentors help them balance life outside the campus as many students had not discussed their private lives outside the campus during group sessions. The study by Soklaridis et al., observed similar results in which the students responded that individual meetings develop a special personal bond between mentors and mentees that results in productive mentoring.[18] Our study showed that the mentors did not play a considerable role in encouraging mentees to take part in co-curricular activities like sports, dramatics, music, arts and photography, cultural society, literary and debating society. Studies have shown that in medical schools there is a positive relationship between active participation in co-curricular activities and academic performance.[19,20] For this reason, it is vitally important for the mentors in our program to encourage students to take part in co-curricular activities. Most of the students showed satisfaction over career counseling provided by their mentors. Literature suggests that mentoring has a huge impact on career choice and development and should be given its due importance in the mentor-mentee relationship.[21,22] In a recent study conducted on faculty of undergraduate medical students, it was seen that mentors play a significant role in the selection of field by undergraduate medical students.[23]

Our results showed that the mentorship program at Shalamar Medical and Dental College has been mostly successful in achieving its goal to satisfy the students. However, according to the students, some aspects need improvement. In the future, we recommend measures to assure the presence of mentors in group sessions as well as their availability to students in case they need individual sessions. It is highly recommended that mentors must be trained before taking on the role.

CONCLUSION

Most of the students (64%) had a positive perception of the mentorship program and the students believed that they benefitted from it. The students felt that the constructive feedback provided by mentors was helpful and it helped them not only in gaining confidence but also in managing time well. It was concluded that to improve the mentorship program, the frequency and duration of meetings should increase.

LIMITATIONS

Data was collected only from 1st year MBBS students, so the results cannot be generalized. We recommend carrying out future research, including students from all the years of medical studies, and also incorporate the perspective of mentors, in order to have a better evaluation of the program and finding out the possible reasons for the mentor's non-availability.

RECOMMENDATION

It is recommended that the mentors should make themselves available for group sessions and promote individual sessions. Moreover, the mentors should be more encouraging towards mentees to make them participate in co-curricular activities and guide them career-wise. Based on these findings, the perception of mentors about this program should also be evaluated to improve the relationship between mentors and mentees.

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Original Article

Hepatoprotective Effects of Apple Cider Vinegar: A Histological Study in Albino Rats

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ABSTRACT

Objectives: To find out the protective effects of apple cider vinegar (ACV) on histology of the liver of albino rats.

Methodology: It was an experimental study conducted at Experimental Research Lab (Animal House) of Post Graduate Medical Institute (PGMI) and Department of Anatomy & Department of Pathology of King Edward Medical University, Lahore. Forty male albino rats were divided by lottery method into 4 groups of 10 animals each. Group 1, control group (CG) was administered food and distilled water 0.5ml/kg body weight. Group 2, the energy drink group (EDG) was given 7ml/kg body weight energy drink. Group 3, apple cider vinegar group (ACVG) was given 2ml/kg body weight apple cider vinegar. Group 4, (ACVG + EDG) was given 2ml/kg body weight apple cider vinegar and 7ml/kg body weight energy drink. All the doses were given by oral gavage once a day for 30 days. Apple cider vinegar was diluted in distilled water in a 1:5 ratio.

Results: Toxicity was produced by oral administration of energy drinks while hepatoprotectivity was observed by co-administration of apple cider vinegar along with the hepatotoxic agent. All slides of control group 1 showed normal architecture of the liver. In group 2, most animals showed a total loss of architecture of the hepatic lobule with congestion and an increase in the diameter of all vessels. Portal triad showed moderate inflammatory infiltrate and nuclei in this group showed pyknotic changes. Animals of group 3 showed dilated central vein with mild periportal inflammation and few dilated vessels in the portal triad. The mean diameter of hepatocytes in this group was 19.90±1.79µm. Routine hematoxylin and eosin (H & E) staining of group 4 showed mild periportal inflammation and few dilated vessels in the portal triad, vacuolization was present, and the mean diameter of hepatocytes was 22.00±1.35µm.

Conclusion: The dose of 1.3ml/150gm body weight/day of energy drink is hepatotoxic to rats at the histological level. Apple cider vinegar when given to rats for a month has a protective role in hepatotoxicity induced by energy drinks in rats as evident by hepatocellular damage at the histological level. Thus ACV offered partial protection to the liver against damage by energy drinks.

Keywords: *Apple cider vinegar. Energy drink. Apple cider vinegar group. Hepatoprotection. Albino Rats.*

INTRODUCTION

Energy drink consumption has become popular in recent years.[1] The consumption around the world has been doubled from the year 2006 to 2012.[2] These drinks are popular among students and athletes and contain glucuronolactone, amino acids mainly taurine, creatine, plant-based guarana, simple sugars like glucose and fructose, various herbs like ginseng and biloba.[1,2] Caffeine is the main ingredient of these drinks which acts as a psychological and physiological stimulant by stimulating the central nervous system.[3] Researchers mentioned that different brands of energy drinks contain different amounts of caffeine.[4] The consumption of such caffeinated energy drinks has shown damaging effects on the levels of liver enzymes in the serum of rats.[5]

Vinegar is an acidic solution that has been in use for different purposes for centuries. Apple cider vinegar (ACV) is the most popular vinegar that has been used by humans. Research has shown positive effects of ACV on many health issues, including high blood pressure, diabetes, skin ailments, heart ailments, high cholesterol, digestive and immune system problems.[6] It is an important detoxifying agent. It oxidizes and thins the blood. Apple cider vinegar neutralizes any poisonous substances that enter the body and kills destructive microbes that may be present in specific foods. A good quality ACV contains amino acids and antioxidants in variable amounts.[7] Acetic acid is present in abundant amounts in ACV along with flavonoids and polyphenols. The healthful benefits of polyphenols are due to their antioxidant, anti-allergic and anti-inflammatory properties.[8]

The liver is the primary organ directly involved in the metabolism of the substances and chemicals entering through the gastrointestinal tract, thus is most vulnerable to the harmful and toxic effects of whatever we eat or drink.[9]

Since the use of energy drinks has become very popular recently, their harmful effects are backed by many studies. An effort is required to find a substance that gives protection to the liver against these drinks. Apple cider vinegar has been shown to have antioxidant properties, thus, it might prove to exhibit a protective effect on the liver against the deleterious effects of energy drinks.[10] So, the present study was designed to evaluate the hepatoprotective effects of ACV on the liver of albino rats.

METHODOLOGY

It was an experimental study conducted on forty adult male albino rats after taking ethical approval from the ethical review board of the institute. The study was

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conducted at Experimental Research Lab (Animal House) of Post Graduate Medical Institute (PGMI) and Department of Anatomy & Department of Pathology of King Edward Medical University, Lahore.

Male albino rats of 8-12 weeks age, weighing 130-160g were divided by lottery method into four groups, with ten animals in each group. Animals of these groups were housed in separate stainless steel cages. The animals were maintained on an ad libitum standard chick diet and distilled water and were allowed to acclimatize for a week before starting the experiment. All animals were weighed before and at the end of the experiment.

Group 1 (CG) animals were administered food and distilled water 0.5ml/kg/day for 30days. Group 2 (EDG) animals were given energy drink in a dose of 7ml/kg body weight[11] that is, 1.3 ml/150 gm weight of rat. Group 3 (ACVG) animals were given ACV once a day in a dose of 2ml/kg body weight diluted with distilled water at a ratio of 1:5,[12] approximately 0.3ml/150gm weight of rat. Group 4 (ACVG + EDG) animals were given the same doses of both liquids, 2ml/kg ACV & 7ml/kg ED, once a day for 30 days. The doses were given once daily by oral gavage for 30 days in each group.

At the end of the experiment, the animal was anesthetized by chloroform inhalation and sacrificed on the 30th day. The doses were given once daily by oral gavage for 30 days in each group. An incision was made in the midline, exposing the abdominal viscera. The liver was grasped gently with the left hand and pulled out. The portal vein, hepatic artery, and bile duct were ligated after exposure. Once the liver was free from all ligaments and vessels, it was removed from the body. All the specimen were preserved in 10% formalin solution, tissue processing was done in an automatic tissue processor followed by sectioning and hematoxylin and eosin (H & E) staining.

STATISTICAL ANALYSIS

The collected information of the four groups was entered into computer software Statistical Package for Social Sciences (SPSS) version 21 and analyzed through it. All the data was collected on proformas. Post-Hoc Tukey test was applied to observe the mean differences among the groups significantly. A p-value $\leq$0.05 was considered statistically significant.

RESULTS

All slides of control group 1 showed normal architecture of the liver. The portal triad comprised a branch each of the portal vein, hepatic artery, and bile duct. Branch of portal vein was lined with flattened endothelium with wider lumen containing erythrocytes as compared to the branch of the hepatic artery that had thick walls and rounded lumen also lined with flattened endothelium while the bile duct was lined with cuboidal epithelium (Figure 1).

In group 2, most of the animals showed total loss of architecture of the hepatic lobule. All blood vessels showed congestion and an increase in diameter. Portal triad showed moderate inflammatory infiltrate with a predominance of lymphocytes. Nuclei in group 2 showed pyknotic changes (Figure 2).

Histological preparations of animals of group 3 showed dilated central vein with congestion at the center. Mild periportal inflammation and few dilated vessels in the portal triad were also seen (Figure 3).

Animals of group 4 stained with H & E staining showed preservation of general architecture of hepatic lobules having central vein lined with flattened epithelium at the center and radiating cords of hepatocytes of one or two cell thickness. Hepatocyte cords enclosed sinusoids that were lined with continuous flattened epithelium and contained specialized stellate cells called Kupffer cells towards their lumen (Figure 4). Routine H & E staining showed mild periportal inflammation and few dilated vessels in the portal triad. Pyknosis and congestion were also seen in both group 3 and group 4.

Cytoplasmic Changes

Cytoplasmic changes were present in all animals of group 2, 3, and 4 while absent in all of group 1. The overall difference was significant with a p-value <0.001 and groups 3 and 4 also had a significant difference from groups 1 and 2 with a p-value <0.001 (Table 1).

Inflammatory Changes

The inflammatory changes around the portal triad were found in groups 2, 3, and 4 while absent in group 1. In group 2, 50.0% had moderate and 50.0% had severe inflammation. In group 3 all 10 animals had moderate inflammation while in group 4, 40.0% had moderate and 60.0% had severe inflammation. The overall difference was found significant with a p-value <0.001 (Table 2).

Changes Around The Central Vein

Changes around the central vein were found present in all animals of groups 2 and 3 while absent in all animals of groups 1 and 4. Congestion and pyknosis were present in all 10 animals of group 2, 3, and 4 while absent in all animals of group 1. The overall difference and difference between groups were significant with p-values <0.001 (Table 3).

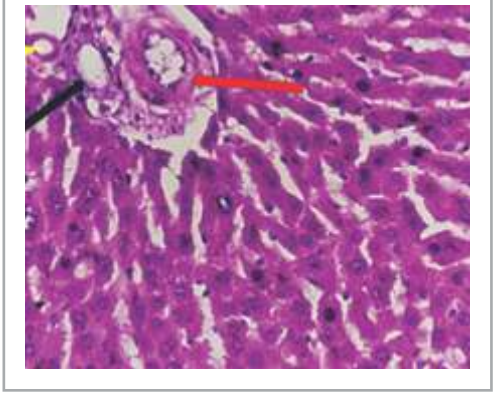

Figure 1: Photomicrograph from Histological Preparation of Rat Liver from Group 1 Showing Cuboidal Cell Lined Bile Duct (Yellow Arrow), Portal Vein (Black Arrow) Lined by Flattened Cells, Hepatic Artery (Red Arrow) Filled with Blood. No Periportal Inflammation seen on H & E Staining (Magnification 40×10 X=400)

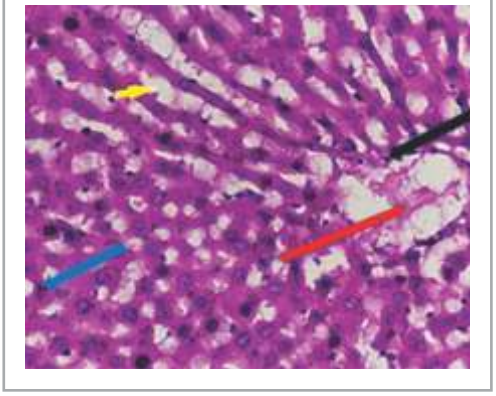

Figure 2: Photomicrograph from Histological Preparation of Rat Liver from Group 2 Showing Dilated Central Vein (Red Arrow) filled with Blood. Lymphocytic Aggregation around Central Vein (Black Arrow). Distorted Sinusoids (Yellow Arrow) with Kupffer Cells are Visible. Pyknotic Nuclei Seen (Blue Arrow) on H & E Staining (Magnification 40×10 X=400)

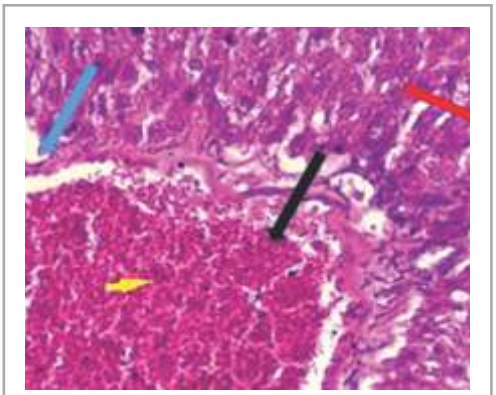

Figure 3: Photomicrograph from Histological Preparation of Rat Liver in Group 3 Showing Congestion (Yellow Arrow) with Dilated Central Vein (Black Arrow), Marked Vacuolization (Blue) with Mild Pyknosis (Red Arrow) is also seen on H & E Staining (Magnification 40×10 X=400)

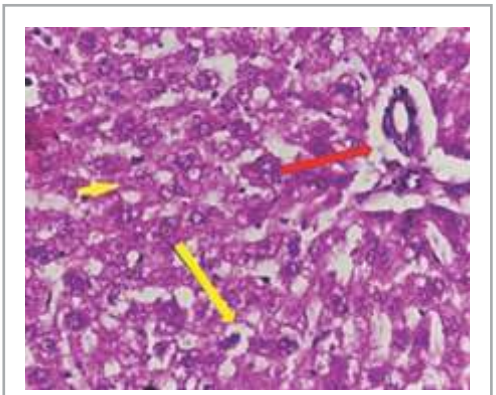

Figure 4: Photomicrograph from Histological Preparation of Rat Liver in Group 4 Showing Reduced Portal Inflammation (Red Arrow) on H & E Staining (Magnification 40×10 X=4)

Table 1: Status and Comparison of Cytoplasmic Changes Among Four Groups

Groups	Cytoplasmic Changes (Vacuolization)					
	Present		Absent		Total	
	n	%	n	%	n	%
Group 1(Control)	0	0.0	10	100.0	10	100.0
Group 2 (Energy Drink)	10	100.0	0	0.0	10	100.0
Group 3 (Apple Cider Vinegar)	10	100.0	0	0.0	10	100.0
Group 4 (ED +ACV)	10	100.0	0	0.0	10	100.0

Table2: Inflammatory Changes around Portal Triad in Study Groups

Groups	Inflammatory Changes Around Portal Triad									
	Absent		Mild		Moderate		Severe		Total	
	n	%	n	%	n	%	n	%	n	%
Group 1(Control)	10	100.0	0	0.0	0	0.0	0	0.0	10	100.0
Group 2 (Energy Drink)	0	0.0	0	0.0	5	50.0	5	50.0	10	100.0
Group 3 (Apple Cider Vinegar)	0	0.0	0	0.0	10	100.0	0	0.0	10	100.0
Group 4 (ED +ACV)	0	0.0	0	00.0	4	40.0	6	60.0	10	100.0

Table 3: Changes around the Central Vein, Congestion and Pyknosis among Four Groups

Groups	Changes Around the Central Vein (n)		Congestion (n)		Pyknosis (n)	
	Absent	Present	Absent	Present	Absent	Present
Group 1(Control)	10	0	10	0	10	0
Group 2 (Energy Drink)	0	10	0	10	0	10
Group 3 (Apple Cider Vinegar)	0	10	0	10	0	10
Group 4 (ED +ACV)	10	0	0	10	0	10

DISCUSSION

Energy drinks are being consumed abundantly all over the world. These drinks are marketed among young people to improve their physical and mental performance such as concentration, attention, and alertness.[5] It is believed to be due to the presence of a combination of different stimulants including caffeine, guarana, ginseng, vitamins, taurine, amino acid derivatives such as carnitine, ribose, carbohydrates, and sugar derivatives, including glucuronolactone.[13,14] Although with their potential beneficial effects, massive consumption of energy drinks has been shown to result in life-threatening toxicity.[7]

The use of energy drinks has been reported to cause damage to the liver tissue in male wister albino rats as mentioned by Khayat et al. and various histological changes indicated the loss of functional integrity and hepatocellular damage to the liver were also seen in this study.[7] There are claims that this hepatocellular damage can be prevented by various herbs and natural liquids like vinegars[15,16], but no scientific evidence was available on hepatoprotection by apple cider vinegar on hepatotoxicity induced by energy drinks. Therefore, the current study was designed to provide scientific proof.

In the present study, changes around the central vein, inflammation around the portal triad, pyknosis, and leukocytic infiltration were observed in our experimental groups after histological damage of the liver by energy drinks (Figure 2, Table 3 & 4). Our findings coincide with the results of Gheith et al., who observed in 2017 that the histological sections of the liver showed architectural distortion after energy drink intake with pyknosis, necrosis leukocytic infiltration, and vacuolization.[4] Similar findings were observed by Harb et at. in 2016 in a case report.[17]

In another study, liver sections of rats treated with energy drinks for 4 weeks were examined that showed leukocytic infiltration and congestion of blood sinusoids. The polyhedral shape of hepatocytes was lost with the appearance of pyknotic nuclei. The normal liver architecture was distorted with marked vacuolization and necrosis of most hepatocytes.[7] These findings coincide with the histological examination of slides of the rat so four energy drink treated group which has shown congestion, vacuolization and inflammation around the portal triad.

In the present study, the histological structure of the liver was damaged by the intake of energy drinks for 30 days. The mean diameter of hepatocytes was observed smallest for group 2 while the largest for group 4 (Table 1). When compared among groups the difference was found significant. Congestion, pyknosis, changes around the central vein, and inflammation around the portal triad was observed in all experimental groups

(Figure 2, 3 & 4). Pyknosis was marked in the energy drink group while marked congestion in the central vein was observed in the ACV group (Table 4). Our findings coincide with the results of another study in which histological sections of the liver showed architectural distortion after energy drink intake at a dose of 3-6ml/day was observed. According to their results, the hepatic lobules were not distinctly demarcated although the hepatocytes were seen to radiate as single plate or cells from the central vein towards the portal tracts. There were moderate intracytoplasmic vacuoles in hepatocytes, and sinusoids were distorted. With high doses of energy drink (6-12 ml/day), hepatocytes increased in size and showed intracytoplasmic vacuolation.[14]

The present study showed lesser pyknosis, congestion, and changes around the central vein in group 4 given ACV and ED (Figure 3 & 4, Table 4). These findings coincide with the results of another study. They reported that the group treated with nicotine and apple cider vinegar in a dose of 2ml/kg body weight showed somewhat normal liver structure and normal appearance of the hepatocytes with decreased vacuolization when compared with the nicotine treated group. Also, the ACV-treated group restored more or less the normal size of the nucleus.[18] Bouazza et al. also observed that animals given apple vinegar showed few inflammatory infiltrates on liver sections.[19]

The current study observed partial protection by ACV to the liver against damage by energy drinks. Further studies are recommended to determine the beneficial as well as harmful effects of different doses of ACV on various organs and systems to determine an appropriate effective dose.

CONCLUSION

The dose of 1.3ml/150gm body weight/day of energy drink is hepatotoxic to rats at the histological level. Apple cider vinegar when given to rats for a month has a protective role in hepatotoxicity induced by energy drinks in rats as evident by hepatocellular damage at the histological level. Thus ACV offered partial protection to the liver against damage by energy drinks.

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