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EDITORIAL

Family Medicine: Indispensable for An Effective Healthcare System

Marie Andrades

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Pakistan is beset with enormous healthcare problems including the quadruple burden of both communicable and non-communicable diseases posing a major public health challenge for all stakeholders[1]. The existing healthcare system consists of three tiers of primary, secondary and tertiary care in the public sector composed of dispensaries, basic health units (BHU), rural health centres, district hospitals, and larger tertiary care hospitals[2]. Despite a well-defined structure of the public health care system, 80% of the general public uses the private sector for healthcare incurring out of pocket expenditure. The low utilization of public services could be driven by multiple factors like deficiency of appropriately trained healthcare personnel resulting in poor quality and poor access, lack of availability of personnel and resources like medicines, cost, and distrust in the government healthcare system. As a result, BHUs are underutilized, whereas the tertiary care system is overwhelmed with patients who could easily be dealt with at the primary care BHU level.

Strong primary healthcare can address up to 90% of a population's health needs. Our BHUs are currently manned by medical officers with no clear training in primary care leading to treatment and management which may be inappropriate for primary care. Family medicine is a primary care specialty geared towards providing ccomprehensive holistic healthcare for the individual and family across all ages, genders, and diseases. It is based on knowledge of the patient in the context of the family and the community, emphasizing not just curative but also preventive, promotive, and rehabilitative healthcare[3]. The trained family physician is able to manage majority of the health problems in the community preventing such patients from requiring tertiary care. A trained family physician acts as a healthcare leader with a role in not just disease management but also in supervision, capacity building of the team, clinical governance, and championing community-oriented primary care.

Correspondence: Professor Marie Andrades. Head, Family Medicine Department, Jinnah Sindh Medical University, Karachi, Pakistan

Email: marie.andrades@jsmu.edu.pk

Strong evidence exists to show the impact of Family Medicine on health systems. Barbara Starfield[4] led a multi-country analysis of health systems concluding that a country's strong primary care/family medicine system is associated with improved population health outcomes for all-cause mortality, all-cause premature mortality, infant mortality, low birth rate, mortality from heart disease, cancer, stroke, improved self-reported health, reduced healthcare spending, and greater patient satisfaction. Further evidence indicates that trained family physicians have a positive impact, on the quality of clinical processes, health services performance, and healthcare team building[5]. Countries where chronic diseases are the principal health burden, family doctors manage 95% of the health problems while absorbing only 5% of the health budget[5].

Realizing the evidence and the need to reduce the burden on tertiary care with greater emphasis on disease prevention, the federal government has incorporated family physicians as an essential element in the health system at primary care level. This is in line with the need to meet the target of the United Nation's 17th Sustainable Development Goal: Good Health[6]. In addition, World Health Organization Eastern Mediterranean Region endorsed a resolution to incorporate Family Practice approach into primary care as a central strategy to achieve Universal Health Coverage[7].

In Pakistan, there are a total of 11,530 Primary Health Care centers with only 18 postgraduate certified family physicians working at these facilities[7]. Currently, less than 2000 doctors are qualified family physicians in the country. Many of them are working abroad primarily due to lack of sufficient jobs for their qualification resulting in an even greater dearth of trained family physicians available to serve the country's needs. Ironically, Pakistan has a total of 108 medical colleges with Family Medicine as a department in only 11 of them and eight Family Medicine postgraduate education sites, of which three are in the public sector.

Recognizing Family Medicine as an urgent priority, Pakistan Medical and Dental Council (PM&DC) has now accepted Family Medicine as a separate specialty with teaching hours mandated in the undergraduate

curriculum emphasizing teaching in the final year of medical school.

Considering the enormous need for trained family physicians in Pakistan, the greatest challenge is overcoming the dearth of qualified human resource. In light of this need, the following solutions are suggested.

There are currently 186,980 doctors registered with PM&DC for their basic medical degree MBBS[8]. Many of them are practicing as general practitioners (GPs) and medical officers (MOs) in the private and public sector providing primary care to the population. Upgrading these GPs and MOs knowledge and skills in Family Medicine through mandatory Continuing Professional Development (CPD) programmes mandated by the PM&DC will help in fulfilling the gap in primary care. These CPD certified physicians can then be developed as Family Medicine trainers to fulfill the dearth of teaching faculty at primary care centers. The centers where they work can be accredited based on fulfilling certain criteria as training sites for undergraduate and postgraduate teaching. The postgraduate trainees will help in overcoming the dearth of human resource for primary care while fulfilling their training needs. These organizations can work with both the government and WHO to provide access to the GPs registered in their database.

Medical colleges should initiate departments of Family Medicine separately or in conjunction with community medicine (where financial resources are constrained) hiring the major and minor diplomas in Family Medicine. Community Medicine and Internal Medicine faculty can also be developed as Family Medicine trainers through training of trainer programmes. Enhancing employment opportunities will help in retention of qualified family physicians within the country.

Short one-year diploma programmes in Family Medicine may be introduced through medical universities for non-practicing doctors (many of whom are women) and practicing GPs to enhance the pool of qualified family physicians. In this regard, three public sector universities have already initiated this step, with Jinnah Sindh Medical University shortly to follow suite.

In conclusion, there is no denying the fact that Family Medicine is the cure for the ailing healthcare system in Pakistan and indispensable for provision of effective primary care in Pakistan's health sector. The dearth of trained family physicians is a stumbling block in this regard. Using a multipronged approach will help in overcoming this challenge.

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ORIGINAL ARTICLE

Statistical Assessment of Drug Release Kinetics and Formulations Attributes of Ranitidine Tablets Available in Karachi, Pakistan

Shaheen Parveen[1], Huma Ali[1], Maria Sodagar[2], Amber Nawab[3], Anum Tariq[2], and Fozia Israr[1]

ABSTRACT

Objective: To elucidate the in vitro equivalence of Ranitidine brands with other quality attributes to provide drug fate for interchangeability or replacement during prescription writing

Methodology: In the present study, quality assessment including range of physico-chemical parameters were evaluated for six selected brands of ranitidine (RT-1 to RT-6).

Result: Results were observed to be in satisfactory points of confinement. Additionally, disintegration profiles of all brands were resolved utilizing phosphate buffer pH 6.8. Information was investigated by factual strategies as recommended by FDA, for example, similarity factor ($f2$) and difference factor ($f1$) and one-way ANOVA technique. Consequences of one-way ANOVA showed no huge variation among the dissolution profiles of reference and test brands.

Conclusion: Correspondingly, results of $f1$ and $f2$ showed similar profiles of test and reference products. In addition, all the brands were found to be best fitted in Weibull model.

Key words: Ranitidine, Weibull model, Dissolution profiles, physico-chemical evaluation, pharmaceutical

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عنوان: پاکستان کے شہر کراچی میں دستیاب RanitidineTablets کی دواسازی اور اسکے اثرات کا شماریاتی جائزہ۔

تعارف: دواسازی میں خاص فعال جُز خاص مقدارِ خوراک میں معیار کے لحاظ سے متغیر ہوتا ہے۔ منہ کے ذریعے دی جانے والی ادویات کے جسم میں جانے کے بعد اثر انداز ہونے کی نوعیت کسی بھی خاص دوا کے معیار کے مستحکم ہونے کی نشاندہی کرتی ہے۔

طریقہ کار: اس تجزیہ میں چھ منتخب کردہ نامی گرامی ادویات کے معیار کا طبّی اور کیمیائی عوامل سے تخمینہ لگایا گیا۔

نتائج: حاصل ہونے والے نتائج اطمینان بخش تھے۔ تمام ادویات کے تحلیل ہونے کا عمل فاسفیٹ بفر پی۔ایچ (٦.٨) کے استعمال سے ہوا۔ اور معلومات کی تحقیق ایف۔ڈی۔اے کے تجویز کردہ شماریاتی طریقوں (Statistical measures) پر کی گئی۔

حاصلِ مطالعہ: f1 اور f2 کے نتائج ایک جیسے تھے۔ تمام ادویات weibull Model میں جانچنے کے لیے بہترین پائی گئیں۔

INTRODUCTION

Ranitidine belongs to H_2 receptor antagonist in pharmacological class of drug, utilized for the management of gastric and duodenal pathological conditions by blocking the acid secretion like in gastroesophageal reflux disease (GERD), peptic ulcer, gastritis, Zollinger-Ellison[1-3]. It is very soluble in water and less permeable to cell membrane. Ranitidine is crystalline in nature, has a whitish to pale yellowish colour with good solubility character in methanol, water, and few organic solvents[4,5]. Ranitidine is categorised in class III drug as defined by BCS recommended via FDA. It is well tolerable and shows atypical interactions and adverse effects and is approximately 50% bioavailable having 300-500 ng/ml serum level with dose of 150 mg observed after 2-3 hours of taken dose and 6% approximately excreted in urine[6,7].

1 Institute of Pharmaceutical Sciences, Jinnah Sindh Medical University, Karachi, Pakistan

2 Faculty of Pharmacy, Ziauddin University, Karachi, Pakistan

3 Faculty of Pharmacy, Jinnah University for Women, Karachi, Pakistan

Correspondence: Prof. Huma Ali, Institute of Pharmaceutical Sciences, Jinnah Sindh Medical University, Karachi, Pakistan

Email: humaali80@live.com

Currently, dissolution test data was used for drug profile comparison. In vitro evaluation of basic drugs plays an important role in bio-waiver assessment and alleviates the regulatory trouble of pharmaceutical industries for product development. UV spectrophotometer was used for analysis of ranitidine samples. Validated method demonstrates that dissolution test is appropriate for the assessment of ranitidine within pharmaceutical solid dosage form during in vitro studies explaining linearity, precision, and accuracy[8-10]. Use of statistical similarity methods helps in conclusion, which is based on concurrence or subjective assessments, but somewhat on scientific facts by controlling predefined maximum error probability i.e. significance limit[11-14].

In developing countries such as Pakistan, where a significant stretch of the population cannot afford to manage the cost of essential medical healthcare services, availability of substandard and spurious pharmaceutical formulations may exacerbate the situation. Studies like ours contribute importantly in prescription writing for alternative drugs at reasonable price. No such study concerning the pharmaceutical equivalence of ranitidine has been conducted in Pakistan. Therefore, this investigation is meant to explain the quality and dissolution effectiveness for correlations of different ranitidine brands available in the market in Karachi, Pakistan.

METHODOLOGY

Sanofi (Pvt) Ltd gifted the ranitidine reference. Sodium hydroxide, methanol, petroleum spirit, and potassium dihydrogen phosphate were used as analytical grade (Merck, Germany). In the present study, reference was chosen as RT-1 product amongst selected brands owing to its excellent physicochemical traits whilst RT-2 to RT-6 were designated as trial/test brands. Hardness tester (OSK Fujiwara, Ogawa Seiki Co. Ltd., Japan), and friabilator (H. Jurgens GmbH and Co., Germany). Thickness, weight, and diameter variation assessments were performed using vernier calliper and analytical balance (AUW-220, UNI Blog, Shimadzu, corp.) Basket Rack Assembly was utilized to perform the disintegration test (Erweka ZT-2 Husenstamn, Germany) (USP, 2003). UV-Visible spectrophotometer (UV-1800, Shimadzu Corp., Japan).

Evaluation for Pharmaceutical Equivalence and Quality Attributes of Ranitidine Tablets

Identification Test (IR Spectrum Technique): An amount was shacked of the powdered tablets containing 25 mg of ranitidine in 5 mL of methanol was soaked for a period of 5 minutes. The filtrate was separated and dried. Petroleum spirit 1 ml was added to the deposit, vessels sides were scratched to incite crystallization, dissipated to dryness. The infrared range of the dried deposit, as per Appendix II, is in good compliance with the reference range of RT[15].

Physicochemical Properties Evaluation: In current investigation, variety of parameters including weight variation, diameter, thickness, hardness, and friability were calculated for the six selected brands of ranitidine (RT-1 to RT-6).

Disintegration Test: Basket rack assembly was used to determine the disintegration time of all formulations. Tablets were introduced in each tube of assembly (N=6) and disintegration was observed. After completion of disintegration of all tablets (when no residue of tablet left on mesh of tube), time was noted in minutes. All brands were tested in similar manner[16].

Assay and Content Uniformity of Ranitidine HCl: Twenty tablets from each brand were accurately weighed. Mean weight of each brand was calculated and ground to powder form. An equivalent quantity of 150 mg of each sample was transferred to volumetric flask of 100 ml, methanol was added to make up the volume and samples were sonicated for 10 minutes and filtered. Filtrate was diluted with methanol to obtain 15 ìg/ml of ranitidine. The absorbance of each sample was observed at 325 nm. Standards were also prepared in the same concentration to calculate the percentage assay of each brand[17]. Content uniformity was also performed in similar way using 10 individual samples of each brand and %RSD values were calculated.

In Vitro Dissolution Study: In addition, RT-1 to RT-6 brands were also estimated for drug release potential by dissolution test. For this, dissolution apparatus II was used at $37^0C + 0.5^0C$; 50 rpm with 900 ml of phosphate buffer pH 6.8. Percentage amount of release contents were measured spectrophotometrically with UV-1800 Shimadzu Corporation Japan. Wave length was 325 nm for the set of experiment.

Comparison of Dissolution Profiles of Different Brands of Ranitidine: Ranitidine reference (RT-1) and test (RT-2 – RT-6) formulations were evaluated by multiple point dissolution method using apparatus II, at 50 rpm speed of rotation in 900 ml of pH 6.8 phosphate buffer. Temperature was adjusted at $37 + 0.5^0C$ throughout the experiment. Samples collection time was up to 120 minutes (5, 10, 15, 25, 30, 45, 60, 90 and 120 min). Ten ml samples were withdrawn at every point of sampling and consequently added with 10 ml fresh medium (previously maintained at 37 +

0.5^0C) in dissolution basket. Drug contents released were approximated by using spectrophotometer at 325 nm.

Ranitidine Release Kinetics

Model-Dependent Method: In current study, various model-dependent and independent tools were applied for the evaluation of drug release patterns of reference and test products. A number of authors have utilized such methods in their investigations to observe release profiles of various drugs (Hanif et al., 2011; Muhammad et al., 2012). Selected models for this study were presented in Table 2. DD-Solver software with Microsoft Excel™ 2007 was used to calculate these model values (Microsoft Corporation, USA). Numerous models were used in this study to analyze the drug release kinetics i.e. *First Order*[11], *Hixson-Crowell cube root law*[12], *Higuchi model*[13] and *Weibull model*[14]as given in Table 2. Model selection criteria were used as adjusted determination of coefficient (r^2), Model Selection Criterion (MSC) and Akaike Information Criterion (AIC).

Application of *Pair-Wise Approach:* Mathematical approaches are widely utilized to compute the profiles of formulations using similarity factor (f_2) and difference factor (f_1). These Pair-Wise techniques are most popular in their application in drug development and design research[14].

Statistical Assessment of Drug Release Kinetics: One-way Analysis of Variance (ANOVA)with Tukey's Post Hoc Test was carried out to conclude the variation in release trends of various brands in phosphate buffer pH 6.8. SPSS 20.0 (SPSS Inc.) was used to perform statistical evaluation.

Table 2: Various Model-Based and Model-independent Equations for Ranitidine Brands Release Kinetics Analysis

MODEL INDEPENDENT TERMS	
Difference factor (f_1)	$f_1 = \left[\frac{\sum_{t=1}^{n}(R_t - T_t)}{\sum_{t=1}^{n} R_t}\right] \times 100$
Difference factor (f_2)	$f_2 = 50 \times log\left\{\left[1 + \left(\frac{1}{N}\right)\sum (Ri - Ti)^2\right]^{-0.5}\right\} \times 100$
MODEL DEPENDENT TERMS	
Hixson–Crowell model	$Q_0^{1/3} - Q_t^{1/3} = K_{HC} \times t$
First Order kinetics	$\text{Log } Q = \text{Log} Q_0 - \frac{kt}{2.303}$
Weibull model	$m = 1 - \exp\left[-\frac{(t-Ti)^{\beta}}{\alpha}\right]$
Higuchi model	$Q = kt^{\frac{1}{2}}$

Physicochemical Attributes: Physicochemical features were estimated by calculating variety of parameters i.e., weight, diameter, thickness, and friability. The results of sample drugs were found to be within the acceptance range when compared with that of the reference. Disintegration, assay, and content uniformity test were performed on sample and reference drugs and results were found to be within range i.e. (within 30 mins), (95%–105%) and (95%–105%) respectively (Table 1). Figure 1 and 2 illustrate the drug release and weight based comparisons of selected brands.

RESULT

Identification Test: Identification test was performed for all the five samples (RT2-RT6) and reference brand (RT1) using IR spectrum technique and the results of the samples were found to be comparable with that of the reference.

Ranitidine Brands (N=20)	Hardness (kg) (N=20)	Thickness (mm) (N=20)	Diameter (mm) (N=20)	Disintegration Time (min) (N=6)	Weight (mg) (N=20)	Assay (%) (N=20)	Dissolution (%) (N=6)
RT-1	9.05 + 0.51	4.37 + 0.14	9.53 + 0.06	8.25	303.68 + 1.54	100.03+2.25	102.88+ 0.79
RT-2	9.81 + 0.52	5.14 + 0.05	8.05 + 0.28	9.00	259.24 + 2.51	99.46+ 1.29	101.07+1.05
RT-3	5.40 + 0.49	3.61 + 0.81	9.62 + 0.12	8.07	239.13 + 2.20	101.98+2.07	103.52+ 0.86
RT-4	9.17 + 0.43	5.51 + 0.12	9.71 + 0.28	3.67	329.88 + 2.13	100.56+2.98	101.69+0.96
RT-5	5.98 + 0.34	4.12 + 0.08	11.26 + 0.11	6.70	369.32 + 2.12	100.12+ 1.33	102.20+ 0.73
RT-6	5.18 + 0.35	4.10 + 0.09	11.33 + 0.15	6.77	297.48 + 2.44	101.73+2.02	103.31+ 0.98

Table 3: Outcomes of Pharmaceutical Equivalence Studies for Reference and Brands of Ranitidine Formulations

Parameters	Specifications	RT-1 (Comparator)	RT-2 (Brand)	RT-3 (Brand)	RT-4 (Brand)	RT-5 (Brand)	RT-6 (Brand)
Identification Test	Complies	Passable	Passable	Passable	Passable	Passable	Passable
Dissolution (%) (n=6)	NLT 80%	Suitable	Suitable	Suitable	Suitable	Suitable	Suitable
Disintegration Test (min) (n=6)	Within 30 minutes	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable
Assay (%) (n=20)	95-105%	Conformed	Conformed	conform	Conformed	Conformed	Conformed
Content Uniformity (%) (n=20)	95-105%	Adequate	Adequate	Adequate	Adequate	Adequate	Adequate

Table 4: Kinetics Evaluation of Ranitidine Brands (RT-1 to RT-6) at pH 6.8

Formulation	**First Order**		**Higuchi**		**Hixson-Crowell**		**Weibull model**		
	r^2	$K_1(h^{-1})$	r^2	K_H $(h^{-1/2})$	r^2	K_{HC} $(h^{-1/3})$	r^2	α	β
RT-1	0.986	0.056	0.779	10.49	0.977	0.011	0.986	15.124	0.946
RT-2	0.973	0.040	0.845	11.35	0.986	0.012	0.988	19.36	1.273
RT-3	0.990	0.045	0.867	10.86	0.994	0.011	0.989	23.42	1.020
RT-4	0.985	0.056	0.769	10.88	0.979	0.012	0.984	22.03	1.069
RT-5	0.985	0.067	0.639	10.54	0.925	0.012	0.989	21.65	1.134
RT-6	0.987	0.049	0.814	10.96	0.989	0.011	0.987	25.83	1.080
Model Selection Criteria									
CODE	MSC	AIC	MSC	AIC	MSC	AIC	MSC	AIC	
RT-1	4.091	41.45	1.202	67.46	3.499	46.79	3.968	42.56	
RT-2	3.403	51.48	1.557	68.09	3.980	46.29	4.163	44.64	
RT-3	4.471	39.57	1.707	64.45	4.944	35.32	4.259	41.42	
RT-4	3.974	43.71	1.158	69.05	3.593	47.13	3.860	44.73	
RT-5	4.022	42.64	1.710	72.45	2.283	58.28	4.243	40.64	
RT-6	4.138	42.74	1.373	67.62	4.263	41.62	4.089	43.18	

Dissolution Profile Comparison: Multiple point dissolution method was used to compare dissolution profiles of different brands of ranitidine (RT2-RT6) with the reference brand (RT1) at 325 nm by using pH 6.8 phosphate buffer and the results were found to be within range i.e. (NLT 80%). Table 3 describes the outcomes of pharmaceutical equivalence studies for reference and brands of ranitidine formulations. Table 4 depicts the kinetics evaluation of ranitidine brands (RT-1 to RT-6) at pH 6.8 alongwith model selection criteria. Furthermore, results reveal that Weibull model was found to be best fitted when evaluated on the basis of model selection criteria.

Model-Dependant, Independent, and ANOVA Results: Release profile comparison of the test and reference products was made using ANOVA-one way technique, model-dependent and model-independent techniques using phosphate buffer pH 6.8 as dissolution media. Results obtained from model-independent technique like difference factor ($f1$) and similarity factor ($f2$) showed similarity in the release profile of test products when compared with the reference. (Table 5) Results obtained from ANOVA detected an insignificant variation between the test (RT2-RT6) and reference product (RT1) as the value of p was found to be 0.997 (Table 6).

Table 5: Evaluation of Difference Factor (f_1) and Similarity Factor (f_2) of RT-1 to RT-6

Ranitidine Brands	f_1	f_2	Comments
RT-1 and RT-2	10.86	50.01	Similar
RT-1 and RT-3	6.40	60.47	
RT-1 and RT-4	3.45	72.74	
RT-1 and RT-5	5.67	63.29	
RT-1 and RT-6	4.43	66.04	

Table 6: Statistical Assessment (ANOVA) of Dissolution Profiles of Ranitidine 150 mg Tablets (RT-1 to RT-6)

	Between Groups	Sum of Squares	df	Mean Square	F	Sig.
pH 6.8	Within Groups	831.454	5	166.291	0.190	0.965
	Total	36772.433	42	875.534		
	Between Groups	37603.887	47			
time	Within Groups	0.000	5	0.000	0.000	1.000
	Total	34800.000	42	828.571		
		34800.000	47			

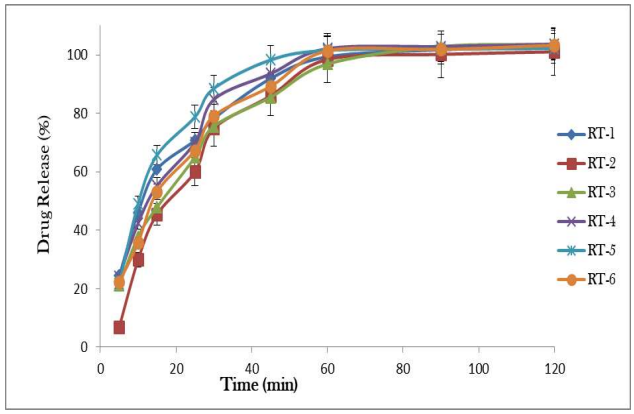

Figure 1: % Drug Release of Ranitidine 150 mg Tablets in Buffer pH 6.8 (RT-1 to RT-6)

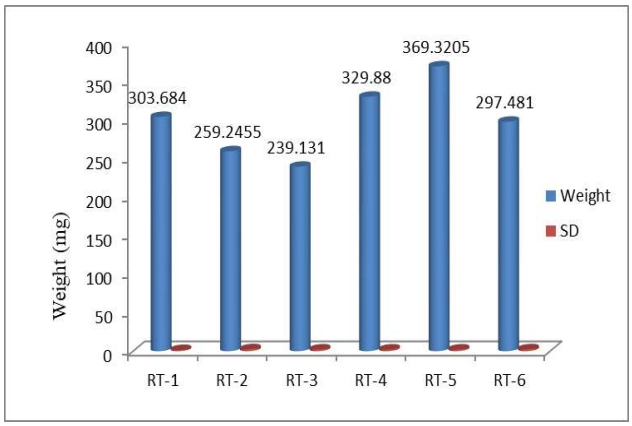

Figure 2: Weight Comparisons of Various Brands of Ranitidine (RT-1 to RT-6)

DISCUSSION

Dissolution test is the way to assess basic parameters, for example, satisfactory bioavailability values and gives necessary information to the formulator in designing of more efficient and restoratively ideal formulations. Dissolution investigation of pharmaceuticals has developed as the absolute most essential test that will guarantee the nature of a product[18]. It is a key explanatory test utilized for identifying physical changes in a functioning pharmaceutical ingredient and in the final product[19]. Pharmaceutical equivalents are defined as indistinguishable measurement forms that contain a similar dynamic fixing i.e. same ester or salt, utilize a similar mode of administration, are interchangeable in concentration and strength and meet the same compendial norms (i.e. identity, quality and purity)[20]. When appropriate, pharmaceutical counterparts must meet a similar substance consistency, disintegration, and dissolution values[21].

In the current study, five commercially available brands of ranitidine (RT2-RT6) were methodically estimated for their physical features and compared with reference brand (RT1) owing to the fact of excellent quality features. Results showed that Hardness (kg) (5.18 ± 0.35 – 9.81 ± 0.52), Thickness (mm) 3.61±0.81 – 5.51 0.12), Diameter (mm) 8.05±0.28–11.33±0.15, Disintegration time (min) 3.67-9, Weight (mg) 239.13±2.20-369.32±2.12, parameters were in acceptable ranges. Result values for assay studies were also found to be in satisfactory ranges (99.46 1.29 – 101.98 2.07). (Table1) Tablets were premeditated for invitro dissolution behaviour for 120 minutes using dissolution apparatus II with 900 ml of phosphate buffer pH 6.8 and the values were found to be in the satisfactory ranges (101.07 1.05-103.52 0.86). (Table 1) In the present study, dissolution profiles of all the brands were evaluated and compared to that of the reference by applying different comparison methods. Techniques applied for comparison were statistical evaluation using ANOVA method, model-independent method including difference factor (f_1) and similarity factor (f_2), model-dependent method. ANOVA followed by the Tukey post hoc multiple comparison test was used to decide statistical worth.

The results showed that the dissolution profiles of the reference and sample drugs were not significantly different as the P value was greater than 0.05 i.e. 0.997 (Table 6).

The mean of the values were used to calculate the difference factor and similarity factor and the results

for $f1$ and $f2$ were found to be in order of (3.45-10.86) and (50.01-72.74), respectively (Table 5). $f1$ values equal to 15 (0-15) and $f2$ range of 50-100 guarantees similarity or proportionality of the two brands and subsequently the sameness of the test and reference[22]. In case, if the estimation of $f2$ is 50, 90% comparability in the profile was shown and the value up to 40, then 80% likeness might be demonstrated. Thus, the outcomes from this investigation uncovered similitude in the medication release.

The slope and coefficient of determination (r^2) values were identified using each model. For First Order and Higuchi models, the r^2 values were in the range of 0.973-0.990 and 0.639-0.867 respectively. Using Hixson-Crowell model, values of r^2 lied in the range of 0.925-0.994. Weibull model gave best curve fitting with highest values of coefficient of determination (0.984-0.989). The determination of the fitting model in the medication discharge behaviour is important to guarantee the viability of the investigation. Different criteria for the choice of the numerical models which depend on the factual treatments are reported in multiple literatures. The most generally utilized strategy uses the coefficient of assurance, r^2 to determine the best fit equation condition. This strategy can be utilized when the parameters of the model conditions are comparable[19,21,22].

Other widely accepted techniques include Model Selection Criteria (MSC) and Akaike Information Criterion (AIC). The AIC, as characterized above, is reliant on the extent of the data points and additionally the quantity of perceptions. What is more, the most fitting model is the one with the littlest estimation of the AIC. The MSC will give an indistinguishable ranking between models from the AIC and has been standardized with the goal that it is autonomous of the scaling of the information focuses. Besides, the most fitting model will be that with the biggest MSC (to boost the "data content" of the model)[24].

As observed from Table 4, Weibull model proves to be the best fit model followed by First-Order, Hixson-Crowell, and Higuchi models. The values of AIC and MSC for Weibull model are in the range of (40.64-44.64) and (3.860-4.259) respectively. AIC values for First-Order, Hixson-Crowell, and Higuchi models are found to be (39.57-51.48), (35.32-58.28) and (64.45-72.45), respectively. Hixson-Crowell model gave MSC value in the range of (2.228-4.944) whereas the MSC values observed for First Order and Higuchi model are in the range of (3.403-4.471) and (1.202-1.710) respectively. Other investigations conducted by Ali et al. and Naqvi et al. also reported Weibull as prominent model for description of drug release of Gatifloxacin tablets[22,23].

CONCLUSION

All the selected products (RT-1 to RT-6) of ranitidine brands verified the adequate physico-chemical characteristics and confirmed the satisfactory in vitro drug release profiles. Such studies not only offer exceptional avenues for choice of superior alternatives accessible in drug market as prominent products but also assist in the most favourable care of patients in developing countries, where ease of access and affordability of these products influence swift healthcare provision.

Authors'contributions: Prof. Huma Ali conceived the idea, worked on data collection, data analysis and review, and also worked on introduction and discussion. Dr Shaheen Parveen and Dr Fozia Israr worked on literature search, results, and discussion. Dr Maria Sodagar and Dr Amber Nawab reviewed the literature, worked on discussion and edited the manuscript. Dr Anum Tariq reviewed the literature, result and conclusion in the discussion. All authors contributed to the final manuscript.

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ORIGINAL ARTICLE

Genetic Variability of Omentin-1 Gene in Apparently Healthy Population

Shazia Nazar[1], Ambreen Qamar[1], Sara Rafique[1], and Shayan Zufishan[2]

ABSTRACT

Objective: Omentin-1 is a recently identified adipokine, highly expressed in visceral omental tissue with anti-inflammatory, antiatherogenic, and antidiabetic properties. Several studies have reported the association of Omentin-1 gene +326 A/T variant with different diseases such as type 2 diabetes, coronary artery disease, rheumatoid arthritis, psoriasis, and breast cancer in different populations. Therefore, the present study was designed to assess the frequency of omentin-1 gene +326 A/T variant in the apparently healthy Pakistani population.

Methodology: This cross-sectional analytical study was conducted at two tertiary care hospitals of Karachi. Participants were recruited from January 2016 to August 2016. The study group comprised of 110 apparently healthy individuals including doctors, nurses, lab technicians, and patient's attendants. Omentin-1 gene +326 A/T variant was determined by polymerase chain reaction-restricted fragment length polymorphism method.

Results: In this study, the wild type genotype (AA) was observed to be 53.6%, mutant genotype (TT) was found to be 6.4%, while mutant genotypes (AT) was found in 40 %. However, the frequency of (AT) mutant genotype was found in 75% males.

Conclusion: The prevalence of AT genotype in 75% of apparently healthy males is a significant finding of the current study. This data may help in the evaluation of population-based risk factors for a number of diseases associated with Omentin-1 gene +326 A/T variant.

Key words: Adipose tissue, Genotype, Omentin-1, Polymorphism

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عنوان: بظاہر صحت مند نظر آنے والے افراد میں omentin-1 جین کا جینیاتی تغیّر۔

تعارف: اس تحقیق کا مقصد پاکستان کے بظاہر صحت مند نظر آنے والے افراد میں omentin-1 +326 A/T جین کی موجودگی اور اس خاص جین کے مختلف پیرامیٹرز کے ساتھ تعلق کی جانچ کرنا ہے۔اس سے حاصل ہونے والی معلومات کا دنیا کے مختلف آبادیوں سے موازنہ بھی اس تحقیق کا حصّہ ہے۔

طریقہ کار: کراچی کے دو بڑے تیسرے درجے کے تدریسی ہسپتالوں میں جنوری 2016 سے اگست 2016 کے دوران یہ تحقیق کی گئی۔جس میں بظاہر صحت مند نظر آنے والے ڈاکٹرز، نرسز، لیب ٹیکنیشنز اور تیمارداروں پر مشتمل 110 افراد کو انکی اجازت سے شامل کیا گیا۔ Omentin-1 gene polymorphism کا تخمینہ PCR-RFLP طریقے سے کیا گیا۔

نتائج: مشاہدات میں genotype AA کی مقدار 53.6%، genotype TT 6.4% اور genotypes AT 40.0% ریکاڈ کی گئیں۔جبکہ مرد حضرات میں AT mutant genotype کی مقدار 75% ریکاڈ کی گئی۔ AT genotype اور triglycerides levels اور waist circumference کے ساتھ خاندانی دل کے امراض کے درمیان مثبت تعلق پایا گیا۔

حاصلِ مطالعہ: مرد حضرات میں AT mutant genotype کی 75% مقدار کے نتائج اس تحقیق کی اہم معلومات ہیں۔حاصل ہونے والی معلومات آیندہ نسل میں Omentin-1 جین سے منسلک کئی بیماریوں کے تحفظ میں مددگار ثابت ہوسکتی ہے۔

INTRODUCTION

Adipose tissue is no longer considered to be an inert tissue that just stores excess energy but it serves as a main endocrine tissue with miscellaneous functions and cellular configuration[1]. Adipose tissue is capable of secreting several biologically active hormone like proteins called adipokines[2]. The association of these proteins has been established in various physiological

1 Department of Physiology, DIKIOHS, Dow University of Health Sciences, Karachi, Pakistan

2 Department of Biochemistry, Karachi Medical and Dental College, Karachi, Pakistan

Correspondence: Dr. Shazia Nazar, Assistant Professor, Department of Physiology, DIKIOHS, Dow University of Health Sciences, Karachi, Pakistan

Email: adrshazia@gmail.com

processes including inflammation, hunger, energy metabolism, insulin resistance, immunity, and angiogenesis[3]. There is a greater possibility of developing cardiac disease, diabetes mellitus, and other metabolic disorders with expansion of visceral fats as compared to subcutaneous fats; however, both types of this tissue are involved in production of a unique profile of adipokines[4]. Omentin-1, a 34kDa protein consisting of 295 amino acids, is a newly identified adipokine which is mainly secreted by stromal vascular cells of visceral omental fat[5,6]. Omentin-1 is an anti-inflammatory protein that is highly expressed in epicardial adipose tissue (EAT) around the heart and coronary arteries[7,8]. The expression of omentin-1 in the heart, lungs, ovary, and placenta has also been reported, but its role in these organs is not yet completely known. Omentin protein exists in two forms: omentin-1 and omentin-2; however the major circulating isoform is omentin-1. Its plasma level is 100 ng/ml to 1 ug/ml[8,9]. Omentin-1 has anti-inflammatory, anti-atherogenic, and anti-diabetic properties. It may improve insulin sensitivity in human adipocyte, myocytes, and hepatocytes via activation of AMPK/Akt pathway[10]. Furthermore, in vitro studies have shown that omentin-1 causes inhibition of tumor necrosis factor Alfa (TNFá) induced degradation of IkB and NF-kB (nuclear factor kappa B) activity to reduce inflammation[11]. Omentin-1 serum level is observed to be lower in obese subjects[12] and type 2 diabetic patients[13]. The Omentin-1 gene contains 1269bp, with 8 exons and 7 introns, localized at 1q22-q23 position[14]. In 2007, Val109Asp single nucleotide polymorphism in exon 4 of omentin-1 gene was reported and +326 A/T nucleotide was declared to be polymorphic[15]. Several studies have discussed the association of this single nucleotide polymorphism (SNP) in different diseases such as type 2 diabetes[15], coronary artery disease[20], rheumatoid arthritis[19], psoriasis[9], and breast cancer[17]. The frequency of +326 A/T nucleotide variant has been described from several world populations so, it is quite important to know the prevalence of such a clinically significant gene polymorphism in the healthy population of Pakistan. Current study is the first study, to the best of our knowledge, that has observed the prevalence omentin-1 gene +326 A/T variant in apparently healthy and non-symptomatic individuals of Pakistan. The data will help in future studies in finding other disorders associated with omentin-1 gene +326 A/T variant.

METHODOLOGY

The current study was approved from the ethical review board of Dr. Abdul Qadeer Khan Institute of Biotechnology and Genetic Engineering (KIBGE). A total of 110 healthy individuals were recruited in the Civil hospital Karachi (CHK) and the Karachi Institute of Heart Diseases (KIHD) from January 2016 to August 2016. Sample size was calculated using open epi software. Convenient sampling was used to select the participants. All the selected healthy participants, including doctors, nurses, lab technicians, and patients' attendants, were informed about the methods and significance of study. Written Consents were taken from all participants. Information about age, gender, family history, ethnicity, and past medical history was recorded through proforma. The blood pressure of the patients was measured via defined protocol. Weight, height, and waist circumference were also measured. Weight and height were measured to the nearest kilogram and centimeter, respectively. BMI was calculated by standard formula (kg/m^2). Blood sample was drawn from brachial vein in early morning and transferred to blood collection tube containing anticoagulant EDTA (ethylene diamine tetra acetate).

Subjects with history of infectious diseases in the previous four weeks, taking anti-inflammatory drugs, statins, diabetes mellitus, heart disease, malignancy, known renal or hepatic disorder were excluded from the study.

DNA Extraction: Whole blood was used to extract genomic DNA via salting out extraction method. Nano-drop (Thermo Scientific USA) was used for quantifying the extracted DNA whereas integrity was checked by horizontal gel system by resolving 2 µl genomic DNA samples on 0.8% agarose gel.

PCR Analysis: Polymerase chain reaction[16] was performed using F-primer5-GAGCCTTTAGGCCATGTCTCT-3' and the R- primer 5'- CTCTCCTTCTTCTCCAGCCCAT-3'[15]. Total volume of 50 µl was prepared for PCR, consisting of genomic DNA, 2 units of Taq DNA polymerase, 1.5mM MgCl, 0.2 mM dNTPs, and 1X PCR buffer of pH-8.3. Initial genomic DNA pre-denaturation was done at the temperature of 94°C for 5 minutes. Denaturation phase consisted of 35 cycles at 94°C, annealing phase had 40 seconds at 62°C followed by 60 second extension phase at 72°C. The final extension time was 5 minutes at 72°C. Amplified PCR product of 471 bp was resolved on 2% of agarose gel and visualized by gel doc system (Fig. 1).

Restriction Fragment Length Enzyme Analysis: PCR products were purified and treated with 10U of restriction enzyme AccI (Molecul-on, New- Zeeland) and placed in incubator for 16 hours at 37 ^{o} C. Digested product of PCR was analyzed by Gel documentation system. TT homozygous individuals had shown two

fragments of 274 and 197 bp, AA homozygous individuals had single fragment of 479 bp while AT heterozygous individuals displayed three bands of 471, 294 and 197 bp (Fig 2).

Statistical analysis: Data were stored and analyzed using IBM-SPSS version 23.0. Count with percentages given for gender, ethnicity, genotype, history of diabetes, hypertension, smoking, and family history of heart disease. Spearman rank correlation was used to see the relationship of genotype and studied parameters. P-values less than 0.05 were considered significant.

RESULTS

A total of 110 participants were recruited for the current study. Seventy seven (75%) males while 33 (25%) females with mean age 49.22 ±6.40 years were included in this study. Total 51.8% data was received from Urdu speaking ethnic group, 26.4% was from Pashto speaking, 4.5% were Balochi speaking, and 17.3% belonged to the Punjabi ethnic group. The mean body mass index was 22.90 ±3.48 kg/m^2, whereas, mean waist circumference was 44.68± 9.95 inches (Table 1).

Table 1: Baseline Characteristics of Studied Subjects (n=110)

Characteristics		n	%
Ethnicity	Urdu	57	51.8
	Pashto	29	26.4
	Balochi	5	4.5
	Punjabi	19	17.3
Gender	Male	77	70.0
	Female	33	30.0
Age (Years)	Mean ±SD	49.22 ±6.40	
BMI (Kg/m2)	Mean ±SD	22.90 ±3.48	
WC (inches)	Mean ±SD	44.68 ±9.95	
Exercise	Yes	37	33.6
Smoking	Yes	54	49.1
Family with heart disease	Yes	61	55.5

In the total of 110 samples, frequency of AA, AT and TT genotypes was found to be 53.6%, 40%, and 6.4% respectively, whereas the frequency of A and T alleles was 78% and 22% respectively (Table 2).

Table 2: Frequency Distribution of Genotype (n=110) and Alleles of Omentin-1 Gene

Gene	n	%
AA	59	53.6
AT	44	40.0
TT	7	6.4
Allelic frequency		
A allele		78
T allele		22

The Spearman's Rank Correlation was applied to analyze the correlation between heterozygous mutant AT genotype and studied parameter. It was found that AT mutant genotype positively correlated with waist circumference, serum triglyceride levels, and family history of heart diseases, however, negative correlation of AT genotype was observed with BMI, FBS, and HDL-C but it was not statistically significant. No correlation was found with gender, ethnicity, junk diet, exercise, and smoking (Table 3 and 4).

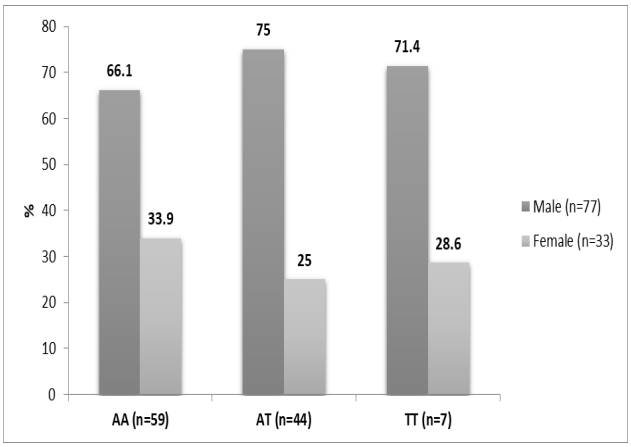
Table 3: Correlation of AT Genotype with Studied Parameters

Parameters	AT Genotype Correlation	p-value
Age, years	0.008	0.93
Waist circumference, inches	0.197	<0.01*
BMI, Kg/m2	-0.010	0.91
FBS mg/dl	-0.088	0.36
T/G mg/dl	0.273	<0.01*
Cholesterol mg/dl	0.040	0.67
HDL mg/dl	-0.032	0.72
LDL mg/dl	0.088	0.36

Quantitative parameters	Genotype Correlation	p-value
Gender	0.086	0.37
Ethnicity	0.124	0.19
Exercise	0.100	0.29
Diet	0.058	0.55
smoking	0.042	0.66
Family history of CAD	0.209	0.02*

*p<0.05 was considered significant for Spearman Rank Correlation

Bar chart 1 is showing the frequency of AA, AT, and TT genotypes in males and females. Seventy-five percent males were found with AT mutant genotype.



Bar chart 1: Genotype distribution of omentin-1 gene with respect to gender

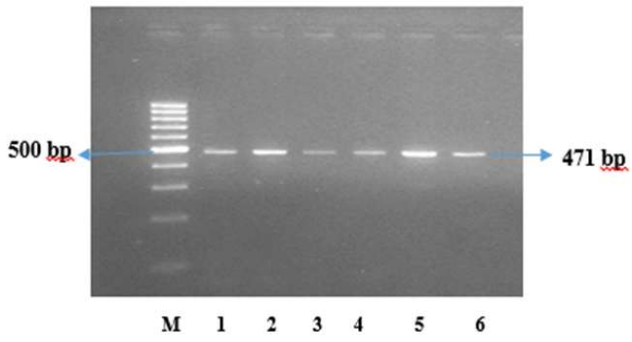


Figure 1| Lane 1-6 PCR product of omentin-1 gene (471 bp)
M = DNA molecular weight marker (100 bp)

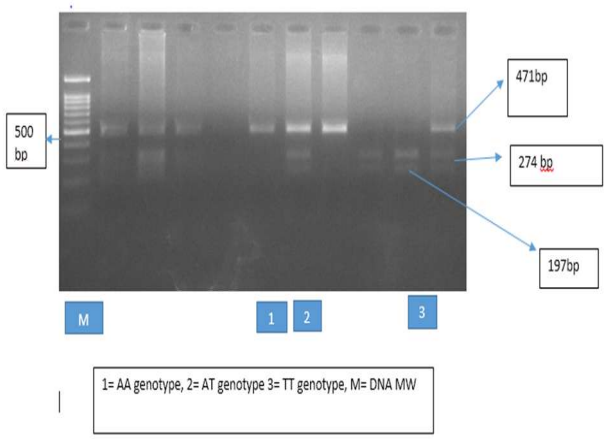


Figure 2. RFLP-PCR analysis

DISCUSSION

In the present study, 110 healthy subjects (33 females and 77 males) were recruited and analyzed for +326 A/T variant in exon 4 of omentin-1 gene. The *omentin-1 +326 A/T* revealed the substitution polymorphism in which there is replacement of A nucleotide by T at position no 326. Allele A is the wild type and allele T is the mutant type. It was found that 59 healthy subjects had wild type homozygous AA genotype, 7 individuals had homozygous TT genotype while the rest of the 44 individuals had heterozygous AT mutant genotype.

The current study revealed the prevalence of 53.6% AA as compared to 40.0% AT genotype in healthy individuals. Surprisingly, AT genotype was found in 75% of male population, so, this data is showing that males are at more risk of developing disorders associated with 326 A/T variant.

While finding correlation between AT mutant genotype with different studied parameters, it was observed that high T/G and waist circumference was statistically associated with AT genotype. Shaffler et al. have revealed the association of AT genotype with T/G in diabetic patients[15], but, we were unable to find any literature which defines this relationship in healthy population. So, our study is the first study that has observed the status of T/G in apparently healthy individuals with AT mutant genotype. No data was found that has stated the association of AT genotype of omentin-1 gene, with waist circumference in apparently healthy individuals.

The current study has observed positive correlation between AT genotype with family history of coronary heart disease. Therefore, this data is determining the fact that subjects with family history of heart disease are at high risk.

While scrutinizing the global publications on omentin-1 gene polymorphism, it was found that the frequency of 'T' allele observed in the present study, was 0.22%, that was close to the result of the study conducted in Turkey (0.20%). Kyrgyz population had shown 0.44%, Iran had 0.28% and 0.37% in two different studies and Caucasian population was found with 0.76% T allele frequency. These results have revealed that the mutant T allele is less prevalent in healthy subjects of various populations. Bahadori et al. have investigated the association of (+326 A/T) polymorphism with breast cancer in Iranian women and reported that TT+AT genotype frequency was 45.6% in cases as compared to 32.7% in control[17]. Boron et al. have identified that genotype AA was more frequent in healthy women of reproductive ages and after menopause, while increased genotype TT was found in unhealthy women of the same age groups[18]. Turan et al., discovered that T allele was high in the psoriatic patients as compared to controls who showed 'A' allele[9]. Yaykasli et al. identified no TT genotype in control group of rheumatoid arthritis[19]. Yourk et al. had identified 2.5 folds increase in TT homozygous mutant genotype in patients with coronary artery disease[20]. These results reveal that overall mutant 'T' allele was found more frequently in diseased subjects as compared to wild type A allele.

CONCLUSION

The objective of the current study was to evaluate the occurrence of the clinically important omentin-1 gene variant (+326 A/T) in apparently healthy population of Pakistan. The most significant finding of this study is prevalence of AT genotype in 75% of male healthy individuals. This gene variant also proved its association with high T/G and waist circumference. So, we may conclude that our apparently healthy population may be future candidates of clinical manifestation related with this gene variant. This data will help in future

studies searching for other disorders associated with omentin-1 gene +326 A/T variant. Further studies are needed to find out whether there is a change in omentin-1 protein physiological function with mutant AT genotype of omentin-1 polymorphism among healthy individuals.

Author's contribution: Dr Shazia Nazar conceived the idea, worked on literature search, data collection, data analysis and review, worked on introduction, discussion and result, drew the conclusion from the discussion and edited the manuscript. Dr Ambreen Qamar and Dr Shayan Zoufishan worked on literature search, results, and discussion. Dr Sara Rafique reviewed the literature, worked on discussion, and edited the manuscript. All authors discussed the results and contributed to the final manuscript.

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ORIGINAL ARTICLE

Impact of Oxidative Stress on Hypertension in Patients on Maintenance Haemodialysis

Sadia Rehman[1], Santosh Kumar[2], Abdul Manan Junejo[2], Fatima Mehboob[3], Hasan Ali[1], and Noorun-nisa Memon[4]

ABSTRACT

Objective: To assess the association of oxidative stress with hypertension and its correlation with the duration of haemodialysis

Methodology: It was a case control study conducted in a public sector tertiary care hospital in 2017. The study participants were recruited from the nephrology ward while the healthy controls were taken from participant neighborhood through frequency matching. Non probability consecutive sampling technique was employed. Cases included were suffering from chronic renal failure and were receiving maintenance haemodialysis. Exclusion criteria was patients suffering from any other chronic illness other than chronic renal failure (pulmonary disease and hepatic insufficiency). Detailed analysis was done with application of ANOVA, Pearson correlation and independent sample t test. P value less than 0.05 was taken as significant.

Results: Highly significant difference was observed in mean serum malondialdehyde, mean plasma superoxide dismutase, mean systolic blood presure, and mean BMI among the cases and controls (p value < 0.001). Positive linear correlation was found between blood presure and serum malondialdehyde i.e. (r = 0.4) while on the other hand strong negative correlation was found between blood presure and plasma superoxide dismutase i.e. (r = -0.73).

Conclusion: Oxidative stress worsens with progression of haemodialysis and leads to development of hypertension.

Keywords: Oxidative stress, serum malondialdehyde, hypertension, plasma SOD, BMI, haemodialysis

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اعنوان: ھیموڈاایلیسس کے مریضوں میں آکسیڈ ٹیو دباؤ کے ہائپر ٹینشن پر اثرات۔

تعارف: آکسیڈ ٹیو دباؤ کا ہائپر ٹینشن اور ھیموڈاایلیسس کے دورانیے کے ساتھ تعلق کی جانچ کرنا۔

طریقہ کار: یہ تحقیق 2017 میں ایک تیسرے درجے کے سرکاری ہسپتال میں ایک خاص کیس کی جانچ کے لیئے کی گئی۔ تحقیق میں حصّہ لینے والے افراد کا تعلق نیفرولوجی وارڈ سے تھا۔ جبکہ انکے مقابلے میں صحت مند افراد قریبی آبادی سے منتخب کیئے گئے, **Chronic renal failure** کی وجہ سے ھیموڈاایلیسس کروانے والے مریضوں پر تحقیق کی گئی۔ تفصیلی تجزیہ کے لیئے **ANOVA, Pearson correlation** اور آزاد سیمپل **t-test** کا استعمال کیا گیا جبکہ (P-value 0.05) لی گئی۔

نتائج: مریضوں اور صحتمند افراد کے درمیان سیرم **Malondialdehyde**، پلازمہ **SOD**، **Systolic BP** اور **BMI** کی اوسط قیمت میں اہم فرق (P-value<0.001) پایا گیا۔ بلڈ پریشر اور سیرم **Malondialdehyde** کے درمیان اثبت خطی تعلق (r = 0.4) پایا گیا جبکہ بلڈ پریشر اور (PSOD) پلازمہ سپر آکسائیڈ dismutase کے درمیان گہرا منفی خطی تعلق (r = -0.73) پایا گیا۔

حاصل مطالعہ: اس تحقیق سے معلوم ہوتا ہے کہ آکسیڈ ٹیو دباؤ، ھیموڈاایلیسس کے ساتھ بڑھ جاتا ہے اور ہائپر ٹینشن کا باعث بنتا ہے۔

INTRODUCTION

The main risk of mortality in patients of end stage renal disease (ESRD) is the cardiovascular diseases[1]. The US renal data system and European Registry of patients, both have highlighted that the incidence of developing a cardiac event is almost five times higher in chronic dialysis patients as compared to the healthy population[2]. Cardiovascular events in ESRD are basically a sequel of hypertension and diabetes as these two are termed number one risk factors for renal failure[3-4]. In chronic renal failure, there is a loss of equilibrium between pro-oxidant and anti-oxidant capacities and a state of increased oxidative stress is evident. The factors which are held responsible for this shift are not only cardiovascular causes but also factors which are more associated to uremia[5-11].

1 Department of Biochemistry, Bahria University Medical and Dental College, Karachi, Pakistan

Department of Nephrology[2] / Department of Physiology[4], Jinnah Sindh Medical University Karachi, Pakistan

3 Department of Medicine, Sharif Medical and Dental College, Lahore, Pakistan

Correspondence: Dr Sadia Rehman, Lecturer, Department of Biochemistry, Bahria University Medical and Dental College, Karachi, Pakistan

Email: dr.sadia89@hotmail.com

The role of oxidative stress is evident in the pathogenesis of hypertension, which has high prevalence in chronic renal failure patients and is considered as the number one risk factor for the progression of cardiovascular diseases[12-13].

Oxidative stress promotes vascular smooth muscle proliferation and deposition of collagen leading to an increase in the intima media thickness ratio and hence causing narrowing of the vascular lumen[12-18]. In addition to this, oxidative stress causes imbalance between the endothelium dependent vascular relaxation and vascular contractile activity by stimulating endothelial injury and decreasing Nitric oxide availability[13-16]. All these factors contribute to the development of hypertension.

Malondialdehyde, a lipid peroxidation end product is produced as a result of the attack by the free radicals on the polyunsaturated fatty acids on the surface of the cell membranes. Malondialdehyde, if produced in high numbers, is a marker of systemic oxidation[14-18].

The term antioxidant refers to a molecule which is capable of stabilizing or deactivating free radicals before they attack normal cells[19]. Endogenous antioxidants are crucial for maintaining optimal cellular functions resulting in systemic health and well-being[20]. However, in conditions promoting oxidative stress, dietary antioxidants may be necessary to maintain the cellular function on optimal levels as endogenous antioxidants may prove to be insufficient.

Glutathione peroxidase, catalase, and superoxide dismutase are the most efficient enzymatic antioxidants. Mitochondria, being the major site of free radical generation, contain a variety of antioxidants present on both sides of their membranes in order to minimize ROS induced stress[21]. Superoxide dismutase is also among the most effective enzymatic antioxidants. Superoxide dismutases (SODs) defend against oxidative damage by enzymatically converting O_2^- to H_2O_2. According to a recent study, SOD is a major antioxidant enzyme in the regulation of oxidative stress during progressive renal injury[22].

When oxidative stress occurs, it triggers the oxidation of molecules such as lipids, proteins, and carbohydrates, leading to lipid peroxidation and accumulation of advanced glycation end products which cause severe damage to the endothelium[11-15]. Moreover, nitric oxide which causes endothelial smooth muscle relaxation is rapidly degraded by the oxygen derived free radical superoxide anion[15-20]. There is also a correlation between oxidative stress and renin activation[32]. Hence oxidative stress is involved in the pathogenesis of various conditions including hypertension, inflammation, and the progression of chronic kidney disease to end stage renal disease[12-16].

The main objective of the study was to assess the association of oxidative stress with hypertension and its correlation with duration of haemodialysis. This study emphasizes on the magnitude and complications of oxidative stress in haemodialysis patients. This study will help the nephrologists to identify oxidative stress as a major causative factor of hypertension and other cardiovascular complications and to change the typical treatment regimes by adding antioxidants to lower the oxidative stress.

METHODOLOGY

A Case Control study was conducted in the Nephrology ward of a public sector hospital of Karachi in 2017. Sample size was calculated by open epi website calculator. (**REFERENCE STUDY:** Locatelli F, Canaud B, Eckardt KU, Stenvinkel P, Wanner C, Zoccali C. Oxidative stress in end-stage renal disease: an emerging threat to patient outcome. Nephrology Dialysis Transplantation. 2003 Jul 1; 18 (7):1272-80.)

A sample size of 90 subjects was calculated which was further subdivided into three groups::

Group A: Healthy control group comprised 30 subjects from the neighborhood who volunteered for the study, and were matched on age, gender, and socio-economic status. Their routine laboratory investigations were within normal ranges.

Group B: Thirty subjects who had been on haemodialysis for up to three years

Group C: Thirty subjects who were on haemodialysis for more than three years

Inclusion criteria for the cases with chronic renal failure was taking haemodialysis therapy for more than two months. Exclusion criteria was the same for all the groups and comprised omission of patients with hepatic insufficiency, chronic pulmonary disease, and diabetes mellitus. Non-probability consecutive sampling technique was utilized for selection of participants. Biochemistry lab investigations and oxidative stress biomarkers were measured in all three groups. Hypertensive cutoff was taken for systolic BP 140 mmHg and for diastolic BP at 90 mmHg. Ethical permission for the present study was taken from the Institutional Review Committee, Jinnah Postgraduate Medical Centre (JPMC), Karachi diary no: NO.F.2-81-IRB/2018- GENL/5173/JPMC. Data which was obtained during the study was kept confidential.

BMI was calculated from weight and height measurements which were obtained through calibrated apparatus available in the wards. Blood pressure was measured in supine position after allowing the participant to relax for 10 minutes. Later on, 10 ml blood samples were collected before dialysis therapy in patients on haemodialysis.

The malondialdehyde (MDA) was estimated in the form of thiobarbituric acid reacting substances (TBARS) by the method of Okhawa et al, 1979. Levels of SOD were measured by using reagent method (method of Kono, 1978).

Data was entered on SPSS version 21. Mean and standard deviation were taken out for all numeric variables, whereas frequencies and percentages were taken out for categorical variables. One-way ANOVA was applied for finding difference in mean between the three groups after fulfilling the assumptions of normality and homogeneity through Shapiro Wilk test and QQ plot and Levenne test. Post hoc analysis was done through Tukey's test. Pearson correlation was applied for finding association of blood pressure with serum malondialdehyde and plasma SOD. Two sample t-test was applied for finding difference in means of serum malondialdehyde and plasma SOD on the basis of gender. P value <0.05 was taken as significant.

RESULTS

A total of n=90 participants were recruited in three groups. Males were n=55 (61%) and females were n=35 (39%). The mean age of the participants was 38±8 years.

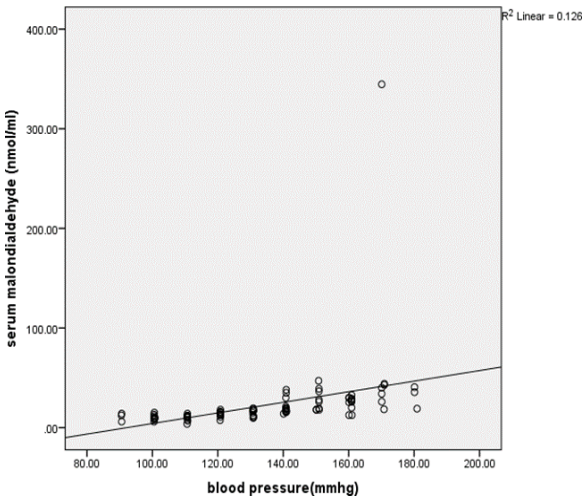
The group A comprised n=30 healthy participants. The mean age of the group was 35±7.7 years. The mean systolic BP was 108±10mmHg. Mean serum malondialdehyde (nmol/ml) was 10.87±3.04. Plasma superoxide dismutase (µ/l) was 108.5±19.4. The mean BMI of the group was 23.4±3.3 kg/m^2.

Group B, n=30 corresponded to participants who were on haemodialysis for the last three years. Mean age of the group was 36±8 years. Mean systolic blood pressure was 136±11mmHg. Mean serum malondialdehyde (nmol/ml) was 15.7±3. Plasma superoxide dismutase (µ/l) was 85±16. The mean BMI of the group was 22.6±3.7.

Group C, n=30 corresponded to participants who were on haemodialysis for more than three years. Mean age of the group was 43±4 years. Mean systolic blood pressure was 159±12mmHg. Mean serum malondialdehyde (nmol/ml) was 31±8. Plasma superoxide dismutase (µ/l) was 46±19. The mean BMI of the group was 20.8±4.6.

Table 1 summarizes the significant differences which were observed when ANOVA was applied for serum malondialdehyde, SOD, systolic BP, and BMI means difference among the three groups. Post hoc analysis was done by the Tukey HSD test. Significant differences were observed between all groups when mean serum malondialdehyde, SOD, and systolic BP was compared. Borderline significance was observed only in Group A and C when mean BMI was compared in between groups through Tukey HSD.

When serum malondialdyde was associated with systolic BP, very highly significant mild positive linear correlation was seen (r=0.4, p value<0.01) Graph 1.



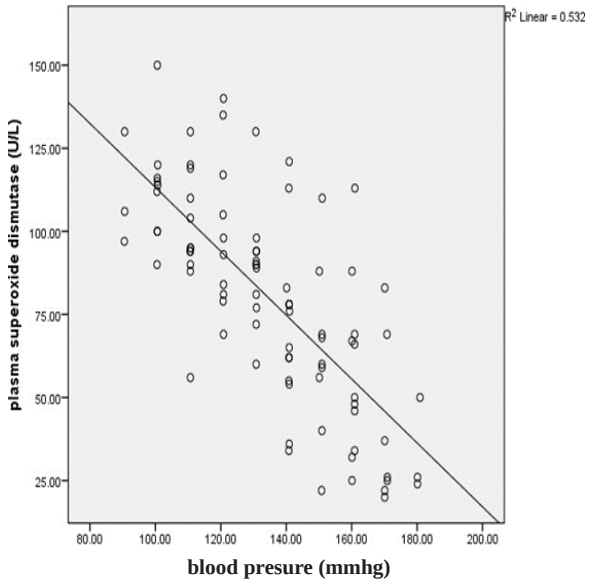
Graph 1: Association of Serum Malondialdehyde and Systolic BP

Table 1: Differences in Mean Numeric Variables Between the Three Groups (N=90)

Groups	n	SerumMalondialdehyde (nmol/ml) Mean±Std.dev	SOD Mean±Std.dev	Systolic BP Mean±Std.dev	BMI Mean±Std.dev
A	30	10.9±3.03	108.5±4	108±10	23.4±3.3
B	30	15.7±2.9	85±16	136±2	22.6±3.7
C	30	31.01±8.48	46±19	159.6±12.3	20.8±4.0
P-Value*	-	<0.01	<0.01	<0.01	0.037

* ANOVA

When superoxide dismutase (SOD) was associated with systolic BP, strong negative linear relationship was observed which was found to be very highly significant (r=-0.73, P value<0.01) Graph 2.



Graph 2: Association of Plasma SOD and Systolic BP

No significant effect of gender was observed on serum malondialdehyde and plasma SOD when independent sample t-test was applied for finding difference among these two parameters on the basis of gender.

DISCUSSION

In uremic patients, cardiovascular disease causes substantially higher morbidity and mortality than in the general population. In dialysis patients, cardiovascular mortality occurs approximately 30 times more than the risk in the general population. Even after stratification for age, gender, and presence of diabetes, this rate remains 10 to 20 times higher. Hypertension is associated with increased oxidative stress. During the comparison of study variables between the controls and patients on haemodialysis, statistically significant difference was found. Significant difference was not found in the mean ages among the three groups compared at 95% confidence interval.

The current study revealed a statistically significant decrease in weight and BMI among cases and controls which is similar to the findings of Larumbe et al[23]. This is in contrast to Araújo et al who reported an increased BMI in females undergoing dialysis[24].

In our study, we found significant increase in mean systolic and diastolic blood pressure of cases as compared to controls, which might be due to ROS induced vasoconstriction and vascular damage, decreased nitric oxide and antioxidant bioavailability, hypervolemia, renin angiotensin system overactivity, erythropoietin administration, and increased sympathetic stimulation. Bansal et al. also reported an increase in mean systolic and diastolic blood pressure in haemodialysis patients[25].

Serum malondialdehyde was significantly increased in group B and group C as compared to controls. This supports our previous findings indicating that oxidative stress in haemodialysis increases as the duration of therapy increases and plays a role in lipid peroxidation leading to development of atherosclerosis and hypertension. The possible mechanism which leads to increase in MDA levels is that ROS activate phospholipase A2 causing peroxidation of many mediators by arachidonic acid which are finally metabolized to MDA. Increased concentrations of malondialdehyde lead to formation of oxidized LDL which plays a major role in the development of atherosclerosis.

Our study further shows that mean SOD levels were significantly decreased in haemodialysis patients as compared to controls. Our results are in accordance with previous studies of Ushanthika et al and Celik et al[27]. Very low levels of SOD were found in group C. The possible mechanism behind this low level depends on several factors, such as age, creatinine clearance, uremic state, dialysis period, selective permeability of the dialyzer membrane to antioxidants and the bacterial contaminants from the dialysate[28]. (Okhawa H et al) In contrast to our study Ninia et al. showed increased levels of SOD in haemodialysis patients[29].

Oxidative stress is a universal challenge in haemodialysis patients. The enhanced oxidative stress status that characterizes haemodialysis patients, mainly occurs because of a diet lacking exogenous antioxidants, accumulation of oxidative products, and loss of antioxidant molecules during haemodialysis. Development of hypertension, chronic inflammation, and CVD mortality is also highly linked to it. It is yet to be made a part of everyday clinical practice even though administering antioxidants appears to be beneficial against oxidative stress development in maintenance haemodialysis patients. Large, prospective studies are urgently needed to elucidate the possible protective role of antioxidant administration against cellular stress that hold the promise to ameliorate the cardiovascular risk profile in CKD and end-stage renal disease. Moreover, the oxidative stress parameters in these patients need to be monitored to avoid the possible outcomes of oxidative stress. Dietary guidelines should also be developed to ensure the intake of adequate vitamins and minerals in these patients.

The strength of our study was the comparison of two groups of dialysis with healthy controls. However, the limitation was the small sample size with selection of study participants through probability sampling technique.

CONCLUSION

The study results have clearly demonstrated a significant increase in oxidative stress marker (malondialdehyde) and a decrease in the antioxidants in patients receiving maintenance haemodialysis as compared to controls. The oxidative stress increases as the duration of dialysis increases showing a positive correlation with hypertension.

Authors' contributions: Dr Sadia Rehman conceived the study, searched for literature, contributed in data collection, analysis and review, and worked on introduction and discussion. Dr Santosh kumar and Dr Abdul Manan worked on literature search, results and discussion. Dr Fatima Mehboob and Dr Hasan Ali reviewed the literature, contributed to the discussion and edited the manuscript. Dr Noor un Nisa reviewed the literature, results and conclusion. All authors contributed to the final manuscript.

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ORIGINAL ARTICLE

Comparative Study of Mean Corpuscular Volume Between Lacto-vegetarian and Non-vegetarian Populations of Tharparkar Village

Suresh Kumar[1], Asma Shaikh[1], Zareen Irshad[1], Vinita Kumari[1], Salma Parween[1], and Shahida Kashif[2]

ABSTRACT

Objective: Anaemia caused due to micronutrient deficiencies is commonly found in our country. Mean Corpuscular Volume (MCV) is the average volume of red cells and largely depends upon micronutrients like B12, folic acid, and iron present in diet. Deficiency of these micronutrient, affects MCV and can cause anaemia. This study aimed to compare the MCV of lacto-vegetarian and non-vegetarian adults living in Tharparkar-Sindh village.
Methods: Case control analytical descriptive study. One hundred apparently healthy strict lacto-vegetarian and non-vegetarian subjects were selected from the same village of Tharparkar. After written consent and preliminary physical examination, blood samples were collected under sterilized condition in two tubes—one containing EDTA for CBC and the second for serum for B12 and folate. Peripheral smear was made at the research field site and fixed with 70% methanol to maintain cellular morphology. Samples were analyzed for complete blood count, serum B12, and folate.
Results: The mean age in vegetarian group was 30.5 years (±8.36) and 30.13(±9.22) in non-vegetarian group. Male to female ratio was 3.4:1. On direct questioning, 54 vegetarians and 24 non-vegetarians agreed to have felt fatigue and lethargy. Most of the subjects in both groups relied on wheat, pulses, vegetables, and milk products as staple food whereas non-vegetarians often consumed eggs and meat as well. In vegetarian group, 83% and in non-vegetarian group, 66% subjects were found to be B12 deficient, while 7% vegetarians and 23% non-vegetarians had low folate level. Nine vegetarians and 22 non-vegetarians were found to be anaemic. A total of 20 vegetarian subjects and 4 non-vegetarian subjects were found with definite high MCV i.e. more than 100.
Conclusion: Majority of the vegetarians and more than half of the non-vegetarians had vitamin B12 deficiency while folate levels were normal in most of the subjects of both groups. MCV is a poor indicator of the severity of B12 and folate deficiency anaemia and normal MCV does not exclude B12 or folate deficiency. Clinicians need to be aware of the low sensitivity of the MCV while screening.

Key words: Anaemia, MCV, Lactovegetarian, Macrocytic

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عنوان: تھرپارکر کے گاؤں کی آبادی کے گوشت خوراور مکمل سبزی خورافراد میں Mean Corpuscular Volume کا جائزہ۔

تعارف: خون کی کمی ہمارے معاشرے میں ایک عام مرض ہے۔ Mean Corpuscular Volume (MCV) ہماری خوراک میں شامل اجزاء جیسے وٹامن B12 آئرن اور فولک ایسڈ پر منحصر ہے۔ اِن اجزاء کی کمی خون کی کمی کا باعث ہوتی ہے۔ اس تحقیق کا مقصد تھرپارکر کے گاؤں میں سبزی خوراور گوشت خورافراد کا MCV کا موازنہ کرنا ہے۔

طریقہ کار: تھرپارکر کے ایک ہی گاؤں سے بظاہر صحت مند نظر آنے والے 100 مکمل سبزی خوراور گوشت خورافراد منتخب کیے گئے۔ اور انکی رضامندی سے حفظانِ صحت کے اُصولوں کو مدّنظر رکھ کر 2 شیشے کی نالیوں میں خُون کے نمونے لیئے گئے۔ ایک ٹیوب کی مدد سے CBC اور دوسری کی مدد سے وٹامن B12 اور folate کی مقدار کا جائزہ کیا گیا۔ نمونوں کی خیلاتی صورت محفوظ رکھنے کے لیئے فیلڈ پر ہی 70 فیصد میتھانول استعمال کی گئی۔ اور انکی CBC، وٹامن B12 اور folate کی جانچ کی گئی۔

نتائج: سبزی خورگروہ کی اوسط عمر 30.5 سال (±8.36) اور گوشت خورگروہ کی اوسط عمر 30.13 سال (±9.22) تھی۔ جبکہ مرد اور عورت کا تناسب 3.4:1 تھا۔ سوال کرنے پر 54 سبزی خوراور 24 گوشت خورافراد نے بتایا کہ انھیں تھکن اور سُستی محسوس ہوتی ہے۔ دونوں گروہوں میں زیادہ تر افراد کی خوراک میں گندم، چاول، دالیں، سبزیاں اور دودھ شامل تھا جبکہ گوشت خورافراد انڈے اور گوشت کا بھی استعمال کرتے تھے۔ سبزی خور گروہ 83% اور گوشت خورگروہ میں 66% افراد وٹامن B12 کی کمی کا شکار پائے گئے۔ جبکہ 7% سبزی خوروں اور 23% گوشت خوروں میں Folate level بھی کم پایا گیا۔ 9 سبزی خورافراد اور 22 گوشت خورافراد خون کی کمی کا شکار تھے۔ اسی طرح 20 سبزی خورافراد اور 04 گوشت خورافراد شدید MCV (100 سے زیادہ) کا شکار تھے۔

حاصلِ مطالعہ: سبزی خوروں میں اکثریت اور گوشت خوروں میں آدھے سے زیادہ افراد وٹامن 12 کی کمی کا شکار تھے جبکہ دونوں گروہوں میں folate level نارمل تھا۔ MCV وٹامن B12 کی کمی اور خون کی کمی کے لیے بری علامت ہے۔ اسکریننگ کے وقت ڈاکٹروں کو MCV کی حساسیت کا خیال رکھنا چاہیے۔

1 Department of Pathology, Sindh Medical College, Jinnah Sindh Medical University, Karachi, Pakistan

2 Liaquat College of Medicine and Dentistry, Karachi, Pakistan

Correspondence: Dr Suresh Kumar, Department of Pathology, Sindh Medical College, Jinnah Sindh Medical University, Karachi, Pakistan

Email: mukhi_suresh@yahoo.com

INTRODUCTION

Tharparkar is a neglected district of the province of Sindh, inhabited by two communities living together for centuries. One of these communities is lacto-vegetarian and the other is non-vegetarian. The lack of basic facilities, education and some religious customs, have placed the population of this area at a high risk of dietary deficiency. Anaemia is defined as a clinical condition characterized by haemoglobin concentration below normal for age, sex, physiological condition, and altitude above the sea level[1]. It is a global concern affecting poor populations in the developing countries mainly due to micronutrient deficiency in their diet. Lack of knowledge of the dietary sources of B-complex vitamin, ignorance of the importance of these vitamins' daily consumption and poor socioeconomic conditions are major contributors of low biochemical levels of these essential nutrients in Pakistani population.

Among other micronutrients, vitamin B12 and folate play the most important role in the maturation of blood cells and stability of neuron cells[2]. Severe and irreversible damage of brain and neuron system are reported to be due to vitamin B12 and folate deficiency[3].

Vegetarians diet is vitamin B12 deficient because the major source of this essential vitamin is animal products while it is found in a negligible amount in dairy product. Hence, vegetarians are found to be more deficient in vitamin B12[4]. On the other hand, folate can be found in both animal and plant sources and its deficiency has been rarely reported[5]. Deficiency of vitamin B12 and folate leads to macrocytic anaemia[6].

Vitamin B_{12} and folic acid are essential dietary components for humans, because they are required for DNA synthesis. Hematopoietic cells are especially sensitive to deficiencies of folate and vitamin B12. With derangement of DNA synthesis due to deficiencies of these vitamins, megaloblastic anaemia ensues[7]. Vitamin B12 and folate deficiencies are quite common among Pakistani individuals and may be a leading cause of megaloblastic anaemia in our population[8].

Mean Corpuscular Volume (MCV) is the average volume of red cells. In specimen, MCV is elevated or decreased in accordance with average red cell size i.e. normal MCV indicates normocytic, low MCV indicates microcytic, and high MCV indicates macrocytic anaemia. This is used as a morphological basis of anaemia[9]. The reference range for MCV is 80-95 fl in adults[10]. Macrocytosis is generally defined as an MCV greater than 100fl.

MCV largely depends upon micronutrients like B12, folic acid, and iron present in diet. Deficiency of these micronutrients affects MCV and can cause anaemia[11]. When the peripheral smear indicates megaloblastic anaemia, the most likely cause is vitamin B12 or folate deficiency[12]. Macrocytosis occurs in approximately 3% of general population worldwide[13]. Megaloblastic anaemia is frequently observed in clinical practice in Pakistan. However, this paper is based on a unique comparative study of MCV in two communities only differing in their dietary pattern.

METHODOLOGY

This descriptive cross-sectional study was conducted on n=100 lacto-vegetarian and n=100 non-vegetarian apparently healthy subjects of both genders, in the rural area of Tharparkar in 2012. The study was conducted after receiving ethical approval from the ethical committee of the Dow University of Health Sciences. Subjects were introduced to this project at a local assembly place. Informed consent was taken before enrolling the participants in the study. The literacy rate in this population was 35 % so the project was explained in detail to the uneducated subjects and their thumb impressions or signatures were obtained on the consent form. The subjects aged between 14 and 55 year. Those with strict lacto-vegetarian diet, were grouped as group 1 and non-vegetarians of the same age were included in group 2. Apparently healthy subjects were included and those who were taking multivitamin preparations orally or parenterally, subjects with history of blood transfusion, history of diarrhoea, worm infestation and those aged less than 14 years were excluded from study. After taking aseptic measures, anti-coagulated whole blood samples were collected for assessing multiple parameters of blood i.e. Complete Blood Count (CBC) for Mean Corpuscular Volume (MCV), vitamin B12 and folate level.

Peripheral blood smear was made at the research field site and fixed with 70% methanol, so that cellular morphology could be maintained. The sample slides and tubes were coded with the subject's serial numbers and full names. After collection of required quantity, the samples were stored in two iceboxes having 4°C and 0°C temperatures. Samples were safely transported within 8–10 hours to Dow Diagnostic Research Laboratory, Karachi for analysis using an automated cell counter TAC–alpha (5 parts) for CBC and serum B12 and folate levels. The peripheral blood smear was stained with Leishman stain. Peripheral smear morphology was observed by using conventional microscopy.

Table 1. Comparison of the Mean Value of Serum B12 and Folate Level in Both Groups Using Independent Sample T-test

Group		Deficiency	Mean	SD	P-Value
Vitamin B12 Deficiency<205	Vegetarian n-100	82	147.29	31.78	<0.01*
	Non-Vegetarian n-100	66	127.7	42.71	
Folic Acid Deficiency<2.6	Vegetarian n-100	7	6.72	2.70	<0.01*
	Non-Vegetarian n-100	23	4.41	2.79	

*p<0.05 was considered significant using independent sample t-test

Table 2. Peripheral Smear Morphology Patterns in Research Population

Gender	Morphology	Vegetarian		Non-Vegetarian	
		n	%	n	%
Male	Normocytic/Normochromic	52	76.5	75	94.9
	Hypochromic/Microcyctic	1	1.5	1	1.3
	Macrocytic	15	22.1	3	3.8
Female	Normocytic/Normochromic	27	84.4	16	76.2
	Hypochromic/Microcyctic	3	9.4	5	23.8
	Macrocytic	2	6.2	0	.0

Table 3. Comparative Analysis of MCV in Both Groups

MCV	Groups			
	Vegetarian		Non-vegetarian	
	n	%	n	%
55 -85	9	9.0	39	39.0
85 - 100	71	71.0	57	57.0
>100	20	20.0	4	4.0

*p<0.05 was considered significant using Pearson Chi Square test

The reference ranges for both the genders were used in accordance to the standard guidelines[14]. For morphology, normochromic normocytic picture was considered as non-anaemic. Hypochromia with poikilocytosis and anisocytosis as either iron deficiency or haemoglobinopathy or both; while macrocytosis was taken as vitamin B12 or folic acid deficiency anaemia. Out of two approaches, i.e. kinetic (focusing on production, destruction, and loss) and morphological (based on RBC size), the latter was used as conventionally done by routine haematology analyzer.

RESULTS

The mean age in vegetarian group was 30.5 years (±8.3n=100) with male to female ratio 2.1:1. In non-vegetarians, the mean age was 30.13 years (±9.3n=100) and male to female ratio was 3.4 :1. The literacy rate was 35% in vegetarian group and while it was 22% in non-vegetarians. Common symptoms reported by subjects were weakness and fatigue at 54% in vegetarians and 24% in non-vegetarians. Mean haemoglobin found in vegetarians was 13.5 (±3.2) and 13.3 (±2.4) in non-vegetarians. A total of 9 subjects were found to be anaemic in the vegetarian group and 22 were found to be anaemic in the non-vegetarian group. MCV was measured and data showed that 9 vegetarian subjects and 39 non-vegetarian subjects had MCV < than 85 fl; 20 vegetarian subjects and 4 non-vegetarian subjects showed MCV values > 100 fl. A total of 83% of the vegetarians and 66% non-vegetarians were found to be vitamin B12 deficient i.e. > 205 ng /ml. However, only 7% of vegetarians were found to have folate deficiency compared to non-vegetarians who showed 23% folate deficiency i.e. < 2.6 ng/ml.

The mean B12 and folate level in vegetarians was 190.24 and 6.73, and 226.71 and 13.9, respectively in non-vegetarians (Table 1).

DISCUSSION

The present study is unique in the sense that (a) to my knowledge, it was conducted for the first time in a rural area of Pakistan which is inhabited equally by the Muslims and the Hindus; (b) It focused on the principle of strict vegetarianism which is the chief feature of the studied Hindu population and non-vegetarians which are the Muslim population; (c) It explored the consequences of vitamin B12 and folate deficiencies on MCV of both groups.

In our study, the mean age of the vegetarians who were found to be vitamin B12 deficient was 30 years, this is different from the study done by Hashim and Tahir in Pakistan in 2006 who found the mean age to be 55 years[14]. In this study, male to female ratio was 2.1:1 in vegetarians and 3.7:1 in non-vegetarians. This difference in genders is due to the practice of veiling of the female population in Tharparkar village. Although all subjects (vegetarian and non-vegetarian) recruited in the study were apparently healthy and did not present any signs and symptoms, but on direct questioning, 54% vegetarians reported history of fatigue and weakness, while 24 % of non-vegetarians reported having unexplained lethargy. These findings are similar to the study done by Stable and colleagues in 1990[15]. These workers suggested that fatigue and lethargy are the most initial symptoms experienced by those who developed macrocytic anaemia. The mean B12 levels were found to be 190.2 (normal 205 ng/ml and above) in vegetarians and 226.7 in non-vegetarians which also showed a preponderance of B12 deficiency among the vegetarian group. The correlation coefficient between B12 and age was positive i.e. 0.173 with significant p value 0.014.

In case of folate level analysis, we also found the most expected results, like only 7% subjects in the vegetarian group and 23% subjects in non-vegetarian group were deficient in folate, with a highly significant p-value less than 0.0001. The mean folate levels were 6.7 ng/ml (normal above 2.6 ng/ml) and 13.9 ng/ml respectively. The correlation co-efficient between folic acid and age was negative i.e. 0.027 with an insignificant p-value 0.70. The prevalence of vitamin B12 deficiency in the present study is much higher than other studies while folate deficiency is also significantly on the higher side.

MCV is the most sensitive index in diagnosis of vitamin B12 /folate deficiency[16]. The degree of anaemia varies but macrocytic and hyper-segmented neutrophils are considered important laboratory findings in many studies[17]. In our studies, we also observed early changes in RBC morphology. The blood films of most of the studied population were found to be normocytic and normochromic (Table 3). These findings matched with the study done by Khanduri and Sharma in 2007[18]. We found that MCV in vegetarian population was 93.6 (±9.9) and 87.6 (±8.2) in non-vegetarians. Maximum range was 118 fl. Seventy-one vegetarians and fifty-seven non-vegetarians had MCV between 85–100 fl in vegetarians with p-value less than 0.001. The most probable cause of borderline increase or definite increase in MCV in vegetarians is vitamin B12 or folate deficiency. This ecological influence on MCV was discussed in the study done by Graves and Lee in 2012[19].

Another intricacy regarding the importance of MCV in vitamin B12/folate deficiency states is addressed in the study done by Spirak in 1982[20]. It says that if iron deficiency and thalassemia coexist with vitamin B12 and folate deficiency, than MCV will not be increased and may decrease in spite of vitamin B12 /and folate deficiency[21]. MCV can increase in many conditions such as in alcoholism, hypothyroidism, liver disease, pregnancy, and reticulocytosis so, we suggest excluding all these condition before labeling and deciding on vitamin B12/folate deficiency[21]. In our study, we had excluded notable conditions just indicated and we found increased MCV and serum vitamin B12/folate deficiency in 20% subjects in vegetarian group and only 4% in non-vegetarian group. Such poor correlation has been reported in the study done by Ward in 2002[23].

CONCLUSION

The vegetarian and non-vegetarian populations of Tharparkar district are sailing in the same boat because of poor economy, harsh environmental and sociocultural conditions. In this study, both groups exhibited severe vitamin B12 deficiency predominately in the vegetarian group, but that was not truly reflected from MCV. It seems to be a poor indicator of the severity of B12 and folate deficiency anaemia, hence, normal MCV does not exclude B12 or folate deficiencies. Clinicians need to be aware of the low sensitivity of the MCV for screening and the upper limit of MCV should not be more than 95 fl.

Authors' contributions: Dr Suresh Kumar conceived the idea, worked on literature search, data collection, data analysis and review, introduction and discussion. Dr Asma Sheikh and Dr Zareen Irshad worked on literature search, results and discussion. Dr Vinita kumari and Dr Salma Parween reviewed the literature,

worked on discussion and edited the manuscript. All authors discussed the results and contributed to the final manuscript.

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ORIGINAL ARTICLE

Knowledge and Frequency of Needle Stick Injury Among Dental Students and House Officers of Bhitai Medical and Dental College, Mirpur Khas

Irfan Ali[1], Faisal Hameed[2], Ali Maqbool[3], Muhammad Kazim[4], Muhammad Aqeel Aslam[5], Saad Uddin Siddiqui[6], and Nabeel Hafeez[7]

ABSTRACT

Objective: To evaluate the knowledge and experience of Needle Stick Injury (NSI) among dental students and house officers at Bhitai Dental and Medical College, Mirpur Khas.
Methodology: This descriptive cross-sectional study was conducted among dental students and house officers. The number of needle stick injuries and post exposure reporting in the past 12 months was assessed by a questionnaire-based survey. SPSS 19 was used to analyze the data.
Result: A total of 330 dental students and house officers participated in this study in which 76.3% were dental students and 23.3% were house officers. As many as 63% had experienced NSI. As many as 31% reported receiving NSI during re-capping of the needles, 21% during administering local anaesthesia injection, and 19% reported getting injured during the scaling procedure. A total of 78.6% of the participants did not report the exposure incident.
Conclusion: The most prevalent of causes reported was receiving injury during the recapping of needle. The reporting of NSIs was found to be insufficient, hence evaded proper follow up because the respondents feared stigmatization and discrimination. Furthermore, awareness was low regarding the means of prevention and protocol.

Key words: Needle stick Injury, Dentistry, Occupational Hazards, Post Exposure Prophylaxis, Blood-borne Pathogens

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عنوان: بھٹائی ڈینٹل اینڈ میڈیکل کالج میرپورخاص کے طلبا اور ہاؤس آفیسرز میں انجیکشن کی سوئی سے ہونے والے انفیکشن کا جائزہ۔

تعارف: اس تحقیق کا مقصد بھٹائی ڈینٹل اینڈ میڈیکل کالج میرپورخاص کے ڈینٹل کے طلبا اور ہاؤس آفیسرز میں انجیکشن کی سوئی سے ہونے والے زخم کے واقعات کی تعداد کا اندازہ لگانا ہے۔

طریقہ کار: اس وضاحتی کروس سیکشنل تحقیق میں ڈینٹل کے طالبعلموں اور ہاؤس آفیسرز سے سوالنامے کے ذریعے یہ معلوم کرنے کی کوشش کی گئی کہ انکے ساتھ گذشتہ 12 مہینوں کے دوران انجیکشن کی سوئی سے زخمی ہونے کے کتنے واقعات پیش آئے حاصل ہونے والے ڈیٹا کا جائزہ لینے کے لیے ایس پی ایس ایس (SPSS)19 استعمال میں کیا گیا۔

نتائج: کُل 330 شرکاء میں 76.3 فیصد ڈینٹل کے طالبعلم اور 23.7 فیصد ہاؤس آفیسر سوالنامہ پُر کیا جس میں سے 63 فیصد نے این ایس آئی کا اعتراف کیا۔ 31 فیصد میں این ایس آئی کی وجہ سوئی کی ری کیپنگ تھی۔ لوکل انستھیسیا انجیکشن سے 21 فیصد اور ڈینٹل اسکیلنگ طریقہ کار سے 19 فیصد ریکارڈ کی گئی۔ 78.6 فیصد افراد نے اس حوالے سے پیش آنے والے واقعے کو رپورٹ نہیں کیا۔

حاصلِ مطالعہ: ڈینٹل کے طلبا اور ہاؤس آفیسرز میں این ایس آئی کی سب سے بڑی وجہ سوئی کی ری کیپنگ تھی جبکہ حاصل ہونے والی معلومات ناکافی تھیں۔ جسکی وجہ شرکاء میں اس بات کا خوف تھا کہ انکے ساتھ امتیازی فرق کیا جائے گا۔ انجیکشن کی سوئی سے ہونے والے انفیکشن سے تحفظ کے لیئے حفاظتی اقدامات کی آگاہی درکار ہے۔

Oral & Maxillofacial Surgery[1] / Department of Oral Biology[3]/ Oral Medicine[5], Bhittai Medical and Dental College, Sciences, Karachi, Pakistan

2 Oral & Maxillofacial Surgery, Sindh Institute of Oral Health Sciences, Jinnah Sindh Medical University, Karachi, Pakistan

4 Senior Lecturer, Department of Prosthodontic, Fatima Jinnah Dental College, Karachi, Pakistan

6 Dental Surgeon, Civil Hospital Karachi, Pakistan

7 Oral & Maxillofacial Surgery, Mohammad Bin Qasim Medical and Dental College, Karachi, Pakistan

Correspondence: Dr Faisal Hameed, Assistant Professor, Oral & Maxillofacial Surgery, Sindh Institute of Oral Health Sciences, Jinnah Sindh Medical University, Karachi, Pakistan

Email: faisal.hameed@jsmu.edu.pk

INTRODUCTION

Dental healthcare workers (DHCWs) are at the greatest risk of exposure to blood-borne pathogens such as hepatitis B, hepatitis C and HIV mostly from percutaneous injury (PI) everyday[1-3]. PI wounds are caused during invasive procedures by accidental cut(s) which break the integrity of the skin, in which NSI is the most common route for blood-borne pathogens[4]. NSI has been reported among healthcare workers since 1980[5].

NSI is the most serious health problem among DHCWs putting their lives and careers at risk[6,7]. One German study stated that the maximum number of participants had experienced NSIs at least once in their profession[8]. A number of published studies have reported the rate of NSI in different populations of DHCWs[9-13]. DHCWs usually face NSIs during dental procedures while using sharp objects and needles[14]. It frequently occurs during local anaesthesia infiltration, re-capping, and disposal of needles or syringe[15]. Among these, re-capping of needles is the most common cause of NSIs[16]. An injury can happen any time during invasive procedures and it can be due to lack of knowledge, lack of training, inexperience, improper handling, and fatigue due to work overload[9,17,18].

In 2002, the World Health Organization (WHO) estimated that up to 2 million healthcare workers experience percutaneous injury each year and are exposed to blood-borne viruses. It also declared that 37.6% of hepatitis B, 39% of hepatitis C, and 4.4% of Human Immunodeficiency Virus (HIV) among healthcare workers globally were acquired through these injuries[19].

In dental schools, the frequency rate of percutaneous injury ranges from 1.97 to 12.5 per 10,000 patient visits[10,20]. According to an observational study by Younai et al, the risk of NSIs among third year students is higher as compared to fourth year students and it is Attributable to lack of experience or improper handling during dental invasive procedures[5,10].

In Pakistan, the rate of NSI is approximately 2.9% in private dental practitioners, 2.4% in undergraduate students, 16.3% in faculty, and 44.7% in house officers. The prevalence rate of transmission for hepatitis C is 3.3% and hepatitis B is 33.3%[21,22].

Although a lot of studies were done on the awareness of NSIs among practicing dentists, little has been done for dental students. The aim of this study was to determine the incidence of NSIs among the 3rd and the 4th year dental students and house officers, to evaluate the level of knowledge, attitude, and management of post-exposure incidents.

METHODOLOGY

This study was conducted at Bhitai Dental and Medical College, Mirpur Khas and included 3rd and 4th year dental students and house officers. It was a descriptive cross-sectional study. The number of NSIs and post - exposure management in the past 12 months was assessed by a questionnaire-based survey. SPSS 19 was used to analyze the data.

RESULT

A total of 330 respondent participated in this study. Out of these, 252 (76.3%) were dental students and 78 (23.6%) were dental house officers. Distribution of male and female students was 24% and 52% respectively and male and female house officers were 14% and 9% respectively as shown in Graph 1.

Figure 1: Distribution of Male and Female participants

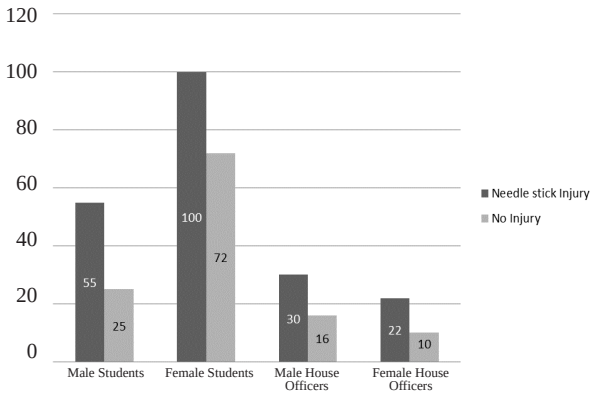

Figure 1: Distribution of Male and Female Participants

In this study, 207 (63%) of 330 had encountered an NSI (Graph 2). Of these, 131 (63.7%) had suffered NSI at least once in a lifetime. Forty-eight (21.7%) were injured twice and 31 (14.9%) were injured more than twice. We also found that only 45 (21.7%) had reported NSIs. Most of the participants (78.6%) did not report NSI to anyone while only 21.4% reported. Only 20.4% received post-exposure management. When participants were asked about hepatitis B vaccination, 73% reported being vaccinated. Sharp bins were used by 27.3% participants and 87% wore gloves during all dental procedures (Table 1).

Among dental students and house officers, NSI occurred most commonly during re-capping (31%) of the needles; local anaesthesia injections accounted for 21% of cases;

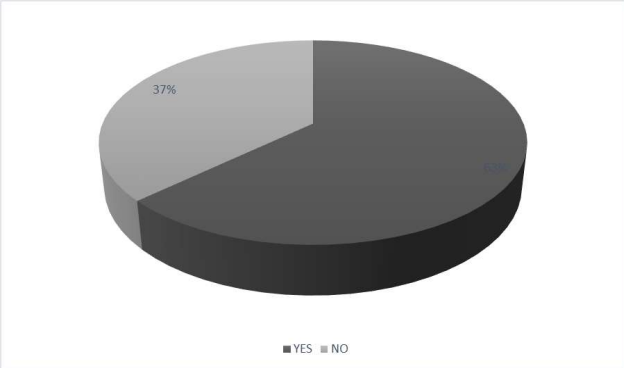

Figure 2: Needle Stick Injury Pie-chart

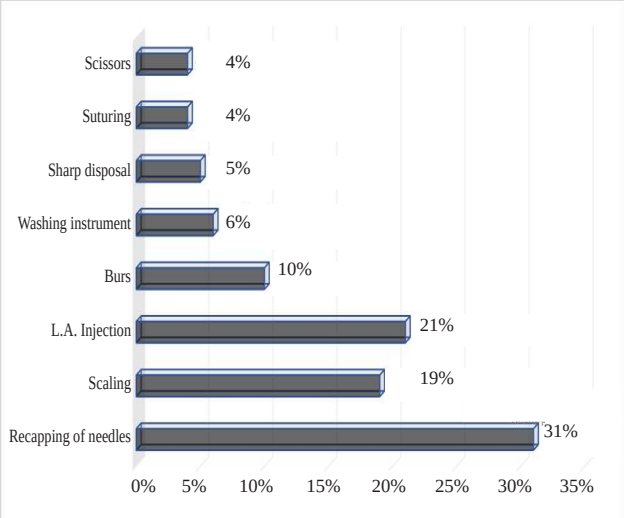

Figure 3: Distribution of Procedures That Cause Needle Stick Injury

Table 1: Prevalence of NSIs, Knowledge, and Attitude

1. How many times during last 12 months?	Once=63.2%, twice 21.7 % more than twice=14.9%	
2. Did you report anyone?	Yes=21.4%	No=78.6%%
3. Did you get the post exposure management?	Yes=20.4%	No=79.6%
4. Do you know the post exposure management protocol?	Yes=38.9%	No=61.1%
5. Are you vaccinated for hepatitis B?	Yes=72.9%	No=27.1%
6. Do you dispose the sharps in the sharp bins?	Yes=27.3%	No=72.7%
7. Do you wear gloves during dental procedures?	Yes=87%	No=13%

and 19% occurred during scaling of teeth. Other reasons reported included the use of instruments such as bur, scalpel, and sharp disposal as shown in Graph 3.

DISCUSSION

NSI is the most common route of transmission for blood-borne diseases among the dental healthcare workers[23]. In our study, 63% students had experienced NSI during different dental procedures, mostly due to inexperience or lack of supervision. According to international studies, inexperience, lack of supervision, excessive workload, and fatigue are the main reasons for NSIs among the dental students[24]. In our study, we found that re-capping of local anaesthesia (LA) injection and dental scaling were the two main causes of NSIs among dental students. Other studies also reveal that sharp needles such as infiltration, block needles, or syringes are the major source of PI among dental professionals[11,25]. In the present study, 48% house officers reported NSIs, citing recapping of the needle as the most common reason. One study in Karachi, Pakistan shows that the risk of NSI is higher in house officers[13]. Other studies have also shown increased rates of NSI in dental postgraduate trainees and dental students[12].

According to Aga Khan University Hospital, Karachi, every year, 12–27% NSIs are reported[26]. One study in Iran has shown that 45% participants had encountered NSI, and the most common reason given was inexperience and lack of knowledge[9].

In dental professionals, the risk of occupational exposures to blood-borne viruses like hepatitis B, hepatitis C and HIV through needle stick injury is a challenging issue[9,27]. In Pakistan, reported frequency of hepatitis B and hepatitis C is 4 to 6%[28]. Aslam M, et al have found that 34% health workers were exposed to hepatitis B or hepatitis C via NSI. Among the blood-borne diseases, hepatitis B is preventable[29]. Worldwide, approximately 80% healthcare workers know about hepatitis B vaccination[30]. In the present study, 70% of the dental students and house officers were found to have been vaccinated against hepatitis B. This affirms that dental professionals should be aware of the threat of NSI and be equipped with protocols regarding the risk of blood-borne infections in their clinical areas. However, 30% dental professional were found not taking NSI seriously. Multiple educational and awareness sessions should be planned to educate them regarding the importance of NSI.

The risk of NSI may be reduced by barriers such as gloves but these are not effective against sharp instruments[31]. In our study, 87% reported wearing gloves during dental procedures as compared to Sumathi Muralidhar[16], who showed that 26% DHCWs were not using gloves. A study in Iran revealed that 96.2 % were using gloves at the time of injury.[9]

In this study, 78.6% did not report the exposure incident fearing stigmatization and discrimination. We also discovered that post-exposure management was completely inadequate in those students who had reported the exposure incident[30].

Reviewing the studies and comparing with our results, we found it extremely important to design, introduce, apply, assure, and reassure compliance to the protocols regarding NSIs in our dental and clinical departments.

CONCLUSION

NSIs are a major route for transmission of blood-borne infections among dental students and house officers, who are aware of this fact. However, reporting of NSIs and post-exposure management was found to be completely insufficient. Preventive protocols should exist in all clinical departments. A dire need exists to invest resources in educating students at the time of their orientation prior to beginning their clinical rotations, stressing the importance of reporting an NSI. Hepatitis B vaccination should be made mandatory in order to minimize the risk of blood-borne diseases. Awareness and educational sessions for blood-borne diseases like HIV, hepatitis B and hepatitis C should be planned for all dental healthcare worker to protect them from these diseases.

Authors'contributions: Dr Irfan Ali and Dr Faisal Hameed conceived the idea, searched for literature search, collected, analysed and reviewed data, wrote the introduction, and discussion. Dr Ali Maqbool and Dr Muhammad Kazim searched for literature, worked on results and discussion. Dr Muhammad Aqeel Aslam and Dr Saaduddin Siddiqui reviewed the literature, worked on discussion and edited the manuscript. Dr Nabeel Hafeez reviewed the literature, result, and conclusion. All authors contributed to the final manuscript.

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ORIGINAL ARTICLE

Self-Image and Its Impact on Academic Performance of Undergraduate Medical Students in Karachi

Rahat Naz[1], Fatima Abid[2], Talat Naz[2], Sohaila Tariq[3], and Sajid Atif Aleem[4]

ABSTRACT

Objective: To evaluate the extent to which students are concerned about their physical appearance and how much self-perception affects them

Methodology: A cross sectional observational study was conducted at the Institute of Health & Business Management and Social Sciences, Jinnah Sindh Medical University (IOH&BMSS), Karachi. A structured online questionnaire was designed and distributed through random sampling among students of medical sciences to collect the data. Data was analyzed using SPSS version 16.

Results: Out of the total of 160 participants in this study, 44 (27.5%) were male while 116 (72.5%) were female. Mean age was 18.24+ 1.68 years while the mean Body Mass Index (BMI) was 21.56+ 4.8. Frequency of depression due to physical appearance found in Grade A and A+ students was 0 while 27.5%, 20.8%, 40.7% was noted among students who got B, B+, B- and 30.4% and 14.3% depression due to physical appearance was noted in C, C- achieving students respectively.

Conclusion: Students who achieved A and A+ grade had no depression due to physical appearance while mild to moderate depression was noted among students who secured lower grades. Furthermore, no relationship was observed between overweight and academic achievements.

Key words: Self-image, academic achievements, students, BMI

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عنوان: کراچی میں طِب کے طلبا کا اپنی شخصیت کے بارے میں ذاتی خیال سے تعلیم پر اثرات کا جائزہ

تعارف: اس تحقیق سے یہ معلوم کرنے کی کوشش کی طلبا اپنی ظاہری جسامت کے بارے میں کس حد تک فکرمند ہوتے ہیں اور انکا خود کے بارے میں ذاتی خیال انکی صلاحیتوں پر کس طرح اثر انداز ہوتا ہے۔

طریقہ کار: اس تحقیق میں استعمال ہونے والے ڈیٹا کو حاصل کرنے کیلئے جناح سندھ میڈیکل یونیورسٹی کراچی کے انسٹیٹیوٹ آف ھیلتھ اینڈ بزنس مینجمینٹ (IOHBM) اور سوشل سائنسز (BMSS) کے طلبا سے آن لائن تفصیلی سوالنامہ پُر کروایا گیا۔ اور اس مواد کو SPSS ورژن 16 پر جانچا گیا۔

نتائج: تحقیق میں شامل ہونے والے مجموعی طور پر 160 طلبا میں سے (27.5%)44 مرد طلبا اور (72.5%)116 طالبات تھیں۔ جنکی اوسط عمریں 18.24 سال(1.68) کم یا زیادہ، جبکہ BMI کی قیمت 21.56(4.8) کم یا زیادہ پائی گئی۔ گریڈ A اور +A والے طلبا میں اپنی شخصیت کے بارے میں ذاتی خیال کی وجہ سے ڈیپریشن کی فریکیونسی صفر تھی جبکہ گریڈ (27.5%)B، (20.8%)B+ اور (40.7%)B- والے طلبا میں ڈیپریشن کی فریکیونسیز بالترتیب 40.7، 20.8، 27.5 فیصد ریکاڈ کی گئیں۔ اور گریڈ C اور -C والے طلبا میں ڈیپریشن کی فریکیونسیز لترتیب۔ 30.4% اور 14.3% ریکاڈ کی گئیں۔

حاصلِ مطالعہ: گریڈ A اور +A والے طلبا میں اپنی شخصیت کے بارے میں ذاتی خیال کی وجہ سے کوئی ڈیپریشن نہیں پایا گیا۔ جبکہ کم گریڈ والے طلبا میں درمیانے درجہ کا ڈیپریشن پایا گیا۔ لہذا اس سے اخذ کیا جاتا ہے کہ جسمانی طور پر موٹا ہونے اور تعلیمی کارکردگی میں قطعی کوئی تعلق نہیں۔

INTRODUCTION

Self-image is a mental representation of one's own personality or is the structured declarative knowledge about one's self that could guide social behaviour. Self-image is the manner in which we take into account our own physical, psychological, and emotional characteristics, the dimensions of our ego and social position. Accurate self-perception is very important to know about attitudinal abilities and personal behavioural knowledge[1]. Teenage is a time in life when structuring one's self-image determines cognitive, affective, and physical development. Self-image stimulates affective qualities to gain behavioural safety and detachment.

Incharge CME and Additional Director HR[1] / Department of Physiology[2] / Department of Research[4], Jinnah Sindh Medical University, Karachi, Pakistan

3 Consultant Sonologist, Mehdi Manji Laboratories, Karachi, Pakistan

Correspondence: Dr Rahat Naz, Incharge CME and Additional Director HR, Jinnah Sindh Medical University, Karachi, Pakistan

Email: rahat.naz@jsmu.edu.pk

Self-image regarding physical appearance becomes more noticeable during teenage years[2]. Hence, self-image has significant impact on students' academic achievements and sometimes there is a need to guide their thoughts regarding their social and emotional behaviour, and also to strengthen the dimensions of ego.

University students are found to have important concerns about body image perceptions because they want to have weight and appearance according to their social preferences[3]. This is because the advertising industry and marketeers use body images to sell their product[4]. Advertisers specifically target teenagers, which raises consciousness about body image in the youth's minds. In turn, teenagers' self-perception of their body image affects their behaviour. It is very important to know and to structure self-perception in a positive direction because poor body image may lower self-assessment that includes self-efficacy and self-esteem, which in turn negatively impacts academic achievements among students. In particular, girls tend to be more dissatisfied with their bodies than boys[5,6].

However, little is known about how undergraduate youth are affected by self-image. Therefore, this research is designed to observe how kinds of body image relate to academic achievement.

METHODOLOGY

The study was conducted at the Institute of Health & Business Management and Social Sciences, Jinnah Sindh Medical University (IOH&BMSS), in Karachi. This cross-sectional observational study included undergraduate students of medical sciences in the age group of 18-35 years. A structured online questionnaire was designed and random probability sampling technique was used to collect data. Data was analyzed using SPSS version 16.

Mean ± Standard Deviation (SD) was calculated for continuous variables like age, etc. Frequency/ Percentages were calculated for all other categorical variables. Level of significance was checked by using Chi-square test.

RESULTS

Table 1 shows the baseline data. Out of the total of 160 participants in this study, 44 (27.5%) were male while 116 (72.5%) were female. Mean age was 18.24+ 1.68 years while the mean Body Mass Index (BMI) was 21.56+ 4.8.

Average Grade Point Average (GPA) of students was 3.0 +0.4. Average level of education attained was 12.48+0.6. The subjects watched TV/day for 3.0+ 5.2 hours and spent about 4.29+6.3. hours per day on social media or internet.

Table: 1 Descriptive Statistics

Gender N=160	Female Male	116(72.5%) 44(27.5%)
		Mean+Std. Deviation
Age (years)		18.24+1.68
Years of Education		12.48+0.6
BMI		21.56+4.8
GPA/Percentage		3.0+0.34
No. of Hours Spent Watching TV/day		3.0+5.2
Time Spent on Internet/ Social Media (Hrs)		4.29+6.3

Body Mass Index (BMI) and Grades of Participants: BMI and grades of all participants are shown in Table 2. A total of 69 (43%) participants had healthy weight. Thirty (19%) participants were overweight and 48 (30%) were underweight.

Most of the participants (44.4%) achieved a "B" grade whereas the least number of participants (1.2%) achieved grades "A and A+". Other grade details are shown in Table 2.

Table 2: BMI and Grades Distribution

	BMI		Grades		
	Frequency	Percentage		Frequency	Percentage
Healthy Weight	69	43	A and A+	2	1.2
			A-	5	3.1
Overweight	30	19	B	71	44.4
			B-	27	16.9
Underweight	48	30	B+	24	15.0
			C	23	14.4
			C-	7	4.4

Impact of Self Image or Physical Appearance:

Relationship between Grades Achieved and Distress Caused by Physical Appearance

Figure 1 shows that high grade achievers (A, A+) did not feel distressed on account of physical appearance whereas those who scored B and B- were found to have mild, moderate, or severe levels of distress concerning their physical appearance. Other lower grade achievers also reported distress ranging from mild to severe due to body image.

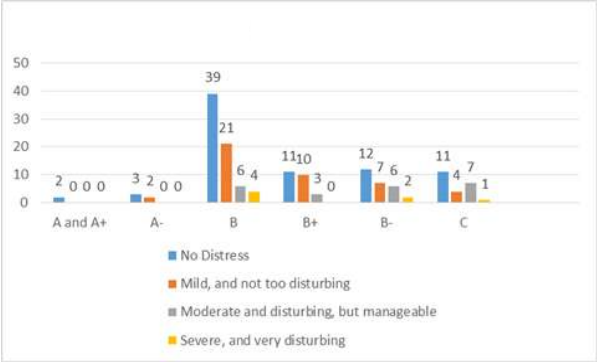

Figure 1: Grades Achieved and Distress

Relationship between Grades Achieved and Concerns about Appearance of Any Unattractive Part of Body

Figure 2 shows students who scored higher grades (A, A+). It shows that 50% were "not at all concerned" whereas the rest were "very concerned" about any perceived unattractive parts of their bodies.

Those who scored A-, B, B-, and C grades were found to have more concerns about their perceived unattractive body parts.

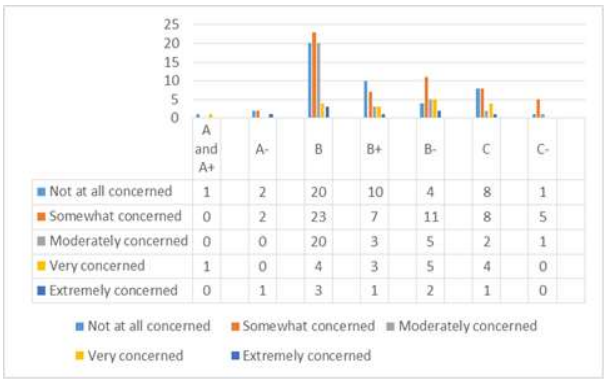

Figure 2: Grades Achieved and Concerns About Appearance

Relationship Between Grades Achieved and Depression Due to Physical Appearance: Table 3 shows that students with higher grades (A, A+) did not get depressed because of their physical appearance whereas those with lower grades reported higher frequency of depression related to their appearance.

Table 3: Grades Achieved and Depression Due to Physical Appearance

Grades	Yes	No	
A and A+	0%	100%	Do you get depressed/ sad/angry about your appearance?
A-	40%	60%	
B	27.5	69.6	
B+	20.8%	75.0	
B-	40.7%	59.3%	
C	30.4%	65.2%	
C-	14.3%	71.4%	

Relationship between Grades Achieved and BMI: Figure 3 showed students with higher grades (A, A+) were overweight whereas most of the other students were of healthy weight.

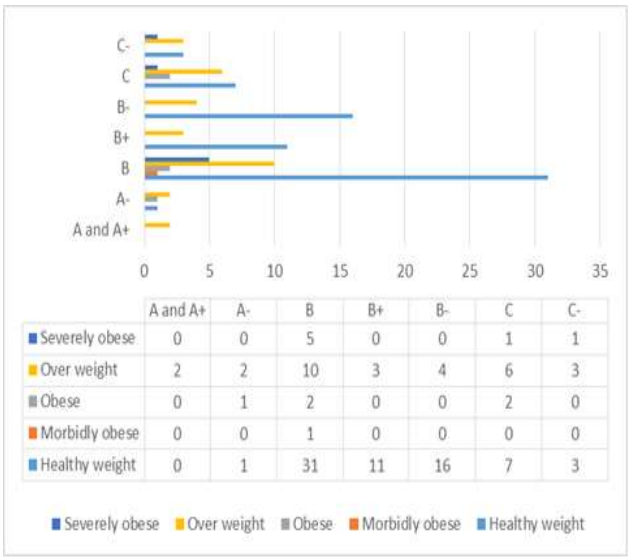

Figure 3: Grades Achieved and BMI

Physical Appearance and Its Impact on Social Life:

Table 4: Problems in Socializing due to Self-image

Gender	Never	Occasionally	Moderately Often	Often	Very often
Male	22	14	4	3	0
	51.2%	32.6%	9.3%	7.0%	.0%
Female	59	32	14	6	3
	51.3%	27.8%	12.2%	5.2%	2.6%

DISCUSSION

Academic grades are considered an important part of progress in education and future job opportunities. In western countries, a lot of research is done to see the impact of self-image on academic performance and their results show that self-image does affect the grades of young people. In youngsters, self-image is an important factor in determining cognitive and affective development. It is important for the stimulation of their spirit, affective qualities, and behavioural security.

This study was conducted in a different culture. In our South Asian society, self-image perception is important, but its impact on academic achievement is not appreciable. The limitation of the study was that it was done in a public sector educational institution where most of the student belonged to middle or lower middle class. For students from these economic backgrounds, their careers are very important and they are required to perform well in studies if they wish to progress in life. It is a matter of survival for them.

Cultural difference is also an important factor. Although some institutions are coeducational, but there is minimum interaction between boys and girls. In a conservative society, most of the females wear either hijab or loose clothes which hide their physique. Table 1 showed a majority of females from the total of 160 participants with only 44 males. Therefore, the general population of the study comprised mainly females with average age around 20 years.

In Table 2, the BMI and grades of all participants are shown. This indicated that most of the participants had healthy weight (43%), while 19% were overweight. Thirty per cent participants were underweight. Most participants had scored 'B' grade (44.4%) whereas 'A and A+' were achieved by only 1.2%.

Figure 1 shows the relationship between the grades and distress due to physical appearance. The high achievers were convinced that physical appearance was unable to impact their grades. Those participants who scored B grades (30%) reported mild disturbance due to their physical appearance.

Figure 3 also confirmed that participants with high grades felt no impact on physical appearance or they managed it very well. The study also showed that those few students who scored A grades were overweight. It can be attributed to cultural differences that students in an eastern society like Pakistan do not appear to be as conscious of their looks as their counterparts in the West, and are able to manage their self-esteem despite being overweight.

The hypothesis is partially supported by the study. Those who scored good grades were the ones who were able to manage the discomfort caused by poor perception of physical appearance. Further studies are required to see the impact of self-image on academic grades in students of private medical colleges which usually enroll students from higher income families, who may be more conscious about their perceptions of self-image.

This study also highlighted the need of physical health and availability of gymnasium and sports facilities for students. The overall health of students is important as a healthy body is required for a healthy mind, which impacts the perception of self-image. It is recommended that educational institutions should have sports facilities where students can take care of their physical health.

Authors' contributions: Dr Rahat Naz conceived the idea, worked on literature search, data collection, data analysis and review, introduction and discussion, reviewed the literature, results, and drew the conclusion from discussion. Dr Fatima Abid worked on literature search, results and discussion. Dr Sohaila Tariq and Miss Talat Naz reviewed the literature, worked on discussion and edited the manuscript. All authors discussed the results and contributed to the final manuscript.

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ORIGINAL ARTICLE

Risk Factors and Frequency of Hypocalcaemia in Unintentional Parathyroid Gland Removal During Thyroid Surgical Interventions

Hurtamina Khan[1], Shireen Ramzan[2], Zahid Mehmood[1], Razzaq Dogar[2], and Javed Jamali[2]

ABSTRACT

Objectives: To identify the risk factors and to determine the frequency of hypocalcaemia in unintentional parathyroid gland removal during thyroid surgical interventions at a tertiary care hospital in Karachi

Methodology: Retrospective study of two years was conducted at Jinnah Postgraduate Medical Centre (JPMC), Karachi from April 2016 to April 2018. The Head & Neck department's records were accessed and analyzed using a proforma.

Results: Total 86 patients were identified. Twenty-four (24) cases from the total of 86 were found to be malignant, which were treated accordingly, resulting in temporary hypocalcaemia in seven (7) cases. Six (6) Cases of Papillary Ca (total thyroidectomy with central neck dissection) resulted in temporary hypocalcaemia in two (2) cases. Seven (7) multinodular goiter (total thyroidectomy) resulted in temporary hypocalcaemia in two (2) cases. In eight (8) cases, lobectomies/ FNAC was done initially, but then their histopathological reports turned out as Papillary Ca. Therefore, completion thyroidectomy with central neck dissection was performed in seven (7) cases and total thyroidectomy with modified neck dissection in one (1) case, which resulted in temporary hypocalcaemia in three (3) cases. Three (3) cases of Follicular Ca in which total thyroidectomy was performed, resulted in no cases of temporary hypocalcaemia.

Conclusion: Re-operated surgeries, malignant thyroid pathology with neck dissection (central/ modified neck dissection) are associated with high chances of unintentional parathyroid gland removal leading to hypocalcaemia. The operating surgeon should consider these risk factors during recurrent malignant surgery with neck dissection.

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عنوان: تھایئرائیڈ غدود کی جرّاحی کے دوران پیرا تھایئرائیڈ غدود کی غیر ارادی طور پر تلفی کی وجہ سے خون میں کیلشیم کی کمی پیدا ہونے کے واقعات اور خدشات۔

تعارف: اس تحقیق کا مقصد کراچی میں تیسرے درجے کے اعلیٰ اسپتال میں تھایئرائیڈ غدود کی جرّاحی کے دوران پیرا تھایئرائیڈ غدود کی غیر ارادی طور پر تلفی کی وجہ سے خون میں کیلشیم کی کمی پیدا ہونے کے واقعات کے تعدد اور خدشات کی جانچ کرنا ہے۔

طریقہ کار: یہ تحقیق جناح پوسٹ میڈیکل سینٹر (JPMC) کراچی میں گزشتہ 2 سالوں میں اپریل 2016 سے اپریل 2018 کے دوران عمل میں آئی۔ ایک پرفارمہ کے ذریعے Head and Neck ڈیپارٹمینٹ کے ڈیٹا کی جانچ کی گئی۔

نتائج: حاصل ہونے والے نتائج میں 86 کیسیز سامنے آئے جن میں سے 24 انتہائی خطرناک صورت میں پائے گئے اور 07 مریض عارضی طور پر خون میں کیلشیم کی کمی کا شکار تھے۔ جبکہ 06 کیسز میں گلے کے درمیان میں کیئے گئے آپریشن جن کے رسولی تھی ان میں سے 02 کو بھی عارضی طور پر خون میں کیلشیم کی کمی کی شکایت تھی۔ اور multinodular goiter کے 07 مریضوں مین بھی 02 کو خون میں کیلشیم کی کمی تھی۔ 08 مریضوں میں جنکو ابتداء میں lobectomies/ FNAC کیا گیا تھا اور ہسٹوپیتھالوجیکل رپورٹ آنے پر رسولی کی تشخیص کی وجہ سے 07 مریضوں کے گلے کے درمیان میں آپریشن کیا گیا اور ایک مریض کے thyroidectomy with modified neck dissection کیا گیا جس کے نتیجے میں 03 مریضوں کو خون میں کیلشیم کی کمی کی شکایت ملی۔ جبکہ Follicular Cain 03 کے مریضوں کو جن کا total thyroidectomy کیا گیا تھا ان میں سے کسی کو بھی خون میں کیلشیم کی کمی کی شکایت نہیں ملی۔

حاصلِ مطالعہ: دوبارا کیا گیا جرّاحی کا عمل اور گلے کے آپریشن (درمیان سے یا موڈیفائیڈ) پیرا تھایئرائیڈ غدود کی غیر ارادی طور پر تلفی کی وجہ سے خون میں کیلشیم کی کمی پیدا ہونے سے براہ راست منسلک ہیں۔ سرجن کو چاہیے کہ موجودہ حالات میں ان تمام خدشات کا خیال رکھے۔

INTRODUCTION

Parathyroid glands were first identified in humans by Ivar Sandstorm[1]. These glands are closely associated with lateral lobes of thyroid and have a short line of embryologic descent but function independently of thyroid gland[2]. Lobectomies and thyroidectomies are the most frequent operations for patients with benign and malignant pathology of thyroid gland/glands[3,4]. Parathyroid injury during unilateral lobectomy is unlikely to result in hypocalcaemia[5]. The operating surgeon should also keep in mind the anatomical variations of the parathyroid glands[6-8]. During thyroidectomies, preservation of the parathyroid glands can be achieved by careful dissection directly on the thyroid capsule separating the parathyroid glands gently from the thyroid capsule[4]. In the 21st century, thyroidectomy has become safe and effective with improved outcomes and minimal morbidity[9,10].

1 Jinnah Postgraduate Medical Centre, Karachi, Pakistan

2 Dow University of Health Sciences, Karachi, Pakistan

Correspondence: Hurtamina Khan, Jinnah Postgraduate Medical Centre, Karachi, Pakistan

Email: drhurtamina@hotmail.com

METHODOLOGY

All 86 patients who underwent surgical procedure were retrospectively included. Out of these, 62 cases were operated for lobectomy and 24 cases were operated for malignant and multinodular goiter. All specimens were sent for histopathology post-operatively, between April 2016 and April 2018 in Head and Neck Department of Jinnah Postgraduate Medical Centre, Karachi.

Proforma was made including age, gender, investigations including ultrasound of the neck, FNAC, CT-Scan contrast, neck/lymph node status, surgical procedure, post-operative histopathological report, serum calcium levels, and follow up.

RESULTS

In our study, 86 patients were included, all of whom had undergone thyroid surgical intervention. Females were 53 (61.62%) and males comprised (38.37%) of the total. Table no. 1 shows more females underwent thyroid pathology than males.

Table No. 1

Gender	Total no. of patients / % of patients
Male	33 (38.37%)
Female	53 (61.62%)

Table no. 2 shows that the pathology of thyroid was benign in sixty-two (62) cases in the two years of the study. In the first year (2016-2017), the number of benign cases was 28 (45.16%) and in the second year of study (2017-2018), 34 benign cases (54.83%) were reported. The rest were malignant thyroid cases which numbered 24 in the two years of study duration: in the first year (2016-2017), 10 cases (41.66%) and in the second year, 14 (58.33%) malignant cases were reported.

Table No. 2

Duration of study	Benign pathology/ Total cases	Duration of study	Malignant pathology/ Total cases
1st year (2016-2017)	28 cases (45.16%)	1st year (2016-2017)	10-cases (41.66%)
2nd year (2017-2018)	34 cases (54.83%)	2nd year (2017-2018)	14 cases (58.33%)

Table no. 3 shows the total surgical procedures performed in both benign and malignant cases of thyroid gland. In 62 cases, lobectomies were performed for benign pathology. In the 24 MNG (multinodular goiter) and malignant cases, total thyroidectomy/ completion thyroidectomy with or without central compartment neck dissection or modified neck dissection was performed. After thyroid surgery, specimens were sent for histopathology conclusion report.

Table No. 5 showed that in total 86 patients, parathyroid gland was unintentionally removed in 09 cases (10.4%) and reported in the histopathological specimen conclusion report. In 77 cases (89.53%), no parathyroid gland was reported to be removed. In 7 patients, hypocalcaemia was clinically positive and was reported.

DISCUSSION

Thyroid surgery is one of the most frequent operations performed in thyroid benign and malignant pathology. Hypocalcaemia is an important complication following thyroid surgical intervention with reports varying from 0.5% to 75%[11,12]. In our study, 9 patients (10.9%) reported with parathyroid gland/tissues in histopathological conclusion report but clinically hypocalcaemia was only positive in 7 patients (8.13%) after post-operative assessment performing Chvostek's sign/ Trousseau's sign.

In our two years of retrospective study, the risk factors identified for unintentional parathyroidectomy are most likely to be the re-operated cases of thyroid which were probably results of fibrosis formation; the malignant thyroid pathology especially in papillary carcinoma of thyroid gland in which completion of thyroidectomy/total thyroidectomy with central neck dissection/modified neck dissection was performed; and the size of thyroid gland in two cases of multinodular goiter in which hypocalcaemia was reported.

Dissection of neck (central neck dissection/ lateral compartments) especially in cases of reoperation is complicated by scarring, fibrosis, and bleeding, making it more prone to injure important structures. Increased awareness when dissecting the central compartment may reduce the risk of unintentional parathyroidectomy[13]. Two recent studies have reported a significant association between inadvertent parathyroidectomy and neck dissection (central compartment clearance and modified neck dissection)[14,15].

Table No. 3: Surgical Procedures Performed in 86 Cases

Benign lesion of thyroid gland	Malignant lesion of thyroid gland + MNG
↓	↓
Lobectomies	Total / completion thyroidectomy With / without neck dissection
↓	↓
62 cases	24 cases

Table No: 4

Lobectomies →	No cases of hypocalcaemia reported
Total / completion thyroidectomy →	7 cases of hypocalcaemia reported
↓	↓
1- 6 cases: Papillary CA + CND	(02)
2- 7 cases: Multinodular goiter	(02)
3- 8 cases: Lobectomies initially but later Histopathology turned out	
CA papillary thyroid + CND	(02)
CA papillary thyroid + MRND	(01)
4- 3 cases: Follicular Ca thyroid (Work-up done for distant metastasis)	(00)

Table No. 5

Histopathological Conclusion Report after Lobectomies/ Total-completion Thyroidectomy/Neck Dissection

↓

Total no of parathyroid glands in Total/ Completion thyroidectomies + Neck dissection

↓

86 cases of Thyroid

↓

77 cases (89.5%) 9 cases (10.9%) reported

However, in our cross-sectional descriptive study, age and sex were found to be irrelevant in carrying any risk for inadvertent removal of parathyroid gland which leads to hypocalcaemia.

Postoperative hypocalcaemia is a major concern after thyroid surgery. The overall incidence of temporary hypocalcaemia among our patients was 8.13%, which reverses spontaneously in most patients after conservative treatment.

The surgeon should keep in mind the anatomical variations of the parathyroid glands to avoid inadvertent injury, revascularization, or resection of parathyroid glands. By knowing the anatomical principles and surgical guidelines, the complication rate of unintentional removal of parathyroid glands during thyroid surgical intervention minimizes.

Re-exploration cases after lobectomy/FNAC conclusion histopathological reports and in recurrence cases,

malignant thyroid pathology with neck dissection, has a strong correlation with unintentional removal of parathyroid glands.

CONCLUSION

Re-operated surgeries after lobectomy and for malignant thyroid pathology with neck dissection are strongly associated with high risk for unintentional removal of parathyroid glands during thyroid surgical interventions which may cause hypocalcaemia in patients. Surgeon should be fully aware and must consider these risk factors during reoperative surgery of thyroid and malignant pathology with central/modified neck dissection.

Authors' contributions: Dr Hurtamina Khan conceived the idea, worked on literature search, data collection, data analysis and review, and the introduction. Dr Shireen Ramzan and Dr Zahid Mehmood worked on literature search, results, and discussion. Dr Razzaq Dogar reviewed the literature, worked on discussion, and edited the manuscript. Dr Javed Jamali reviewed the article. All authors discussed the results and contributed to the final manuscript.

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REVIEW ARTICLE

Phytomedicine: *Silybum marianum* (Silymarin) as an Effective Hepato-protective Source from Nature

Huma Shareef[1], Erum Zaheer[2], Saima Abedin[3], and Hina Hassam[3]

ABSTRACT

Objective: To review the efficacy and safety of milk thistle for management of hepatic disorders
Methodology: A thorough literature survey was carried out for the article. Following key words (*Silybum marianum*, Liver) were used to search the articles on Google Scholar and PubMed. More than 50 articles published between 2000 and 2018 were taken into consideration.
Result: The review explored the main constituents of milk thistle and its properties to protect liver against various toxins, alcohol effects, and viral attacks. Silymarin is capable of regenerating liver and bring the hepatic markers, Alanine transaminase (ALT), Aspartate transaminase (AST) and others, to normal levels. Reported toxicities of silymarin are few.
Conclusion: *Silybum marianum* has proved its higher efficacy and safety in hepatic disorders, as compared to other therapies and therefore is an agent of choice. Furthermore, its cost effectiveness and ability to be developed as various dosage forms, like emulsion and nanoparticles, undoubtedly ensures its extensive use in future.

Key words: Silybum marianum, Liver

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عنوان: نباتاتی دوا(Silymarin) جگر کے امراض سے بچنے کا قدرتی ذریعہ۔

تعارف:اس تحقیق کا مقصد ایک خاص نباتاتی دوا(Silymarin) کے جگر کے امراض کے علاج کے لیئے کرشماتی اثرات کا جائزہ کرنا ہے۔

طریقہ کار: جسکے لیئے گوگل اسکالر پر Key words جگر اور Silybum marianum کو تلاش کرتے ہوئے سال 2000 سے سال 2018 تک اس موضوع پر شائع ہونے والے 50 سے زائد آرٹیکلز کا ادبی سروے کیا گیا۔

نتائج: جائزہ سے ہونے والے انکشافات سے پتا چلا کے (Silymarin) میں پائے جانے والے اجزاء اور اس کی خصوصیات جگر کو کئی مختلف بیماریوں سے محفوظ رکھ سکتا ہے۔اور اسے دوبارہ تقویت بخشتا ہے۔اور اسکی قوّتِ مدافعت میں اضافہ کرتا ہے۔

حاصلِ مطالعہ: (Silymarin) جگر کے امراض کے علاج کے لیئے اپنی نمایاں خصوصیات کی بنا پر دوسری ادویات کے مقابلے میں خود کو بہتر ثابت کرنے کی صلاحیت رکھتا ہے۔جسکی وجہ سے یہ سب سے مشہور اور سب سے زیادہ استعمال ہونے والی نباتاتی دوا ہے۔اور اس کی مدد سے کئی دوسری ادویات بنانے میں مدد ملتی ہے۔مستقبل میں اس کا استعمال بڑھ سکتا ہے۔

INTRODUCTION

Silybum marianum L, commonly known as milk thistle or Marian thistle belongs to botanical family *Asteraceae*. It is an annual or biannual specie found all over the globe especially in the Southern Europe and Asia (Figure I). Milk thistle has been famous since the olden days, to cure hepatic and gall bladder ailments such as hepatitis B and C, liver cirrhosis, cancers, jaundice, and alcoholic and non-alcoholic fatty liver disease. In addition to its traditional uses, it has also clinically proven its efficacy to combat certain poisoning conditions which include snake and insect stings, alcohol as well as mushroom Amanita poisoning[1]. Its role also encompasses the stimulation of biliary secretion and production of breast milk[2]. Chinese traditional medicine and Indian herbal practitioners

Department of Pharmacognosy[1] / Department of Pharmaceutical Chemistry[2] / Department of Pharmaceutics[3], Institute of Pharmaceutical Sciences, Jinnah Sindh Medical University, Karachi, Pakistan

Correspondence: Prof. Huma Shareef, Department of Pharmacognosy, Institute of Pharmaceutical Sciences, Jinnah Sindh Medical University, Karachi, Pakistan

Email: huma.shareef@jsmu.edu.pk

use *Silybum marianum*[3]. Silymarin is an example of a herbal medicinal preparation[4].

Figure 1: Silybum marianum

The fruit of *Silybum marianum* contains lipids, carbohydrates, proteins, phytosterols, and tocopherols[5]. Silymarin is a mixture (lipophilic extract) from the seeds and fruits of *Silybum marianum* which contains silibinin/silybin (50-60%), isosilybin/ isosilibinin (5%), silychristin (20%), and silydianin (10%). Silimonin and isosilychristin are also present in minor quantities. Among these isomers flavonolignans, derivatives of flavanone, silibinin is in a huge amount and is highly active (Figure 2)[6].

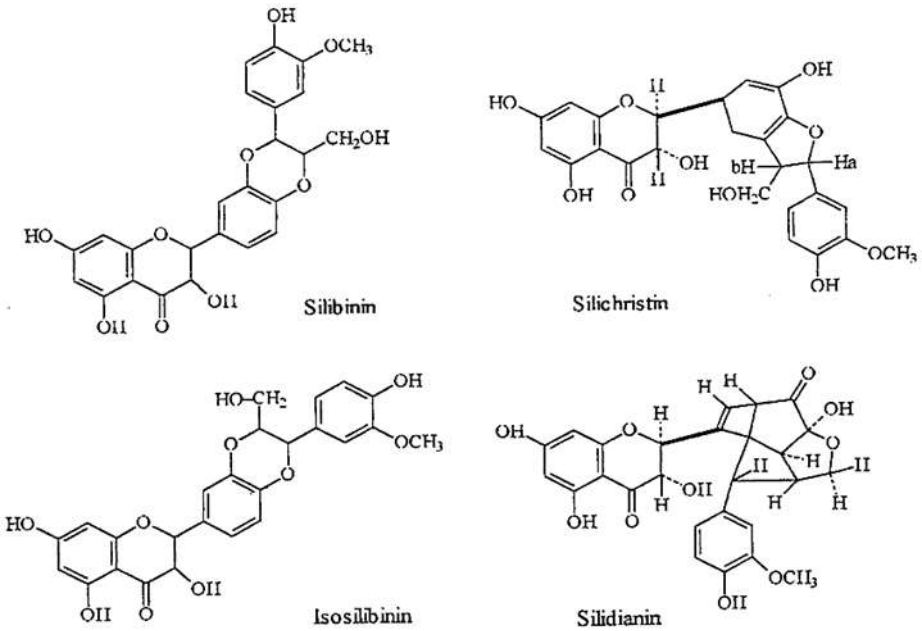

Figure 2: Chemical constituents of milk thistle

The pharmacokinetics of silymarin indicates 20-50% absorption after an oral dose, and 80% excretion in bile. Most studies conducted on silibinin, the main active constituent, confirm its half-life of 6 hours. Following an oral dose, silibinin is excreted 3-8% in urine, 20-40% is metabolized as sulphate and glucuronide derivatives and is found in bile juice. The rest is excreted unchanged in faeces. Peak concentration of silibinin is achieved within 2-9 hours[7].

Silymarin possesses excellent tolerance and has no major adverse drug reactions[8,9]. Due to these attributes several companies have started to market silymarin as a nutritional supplement in USA and Europe. In several studies on laboratory animals, silymarin has been found nontoxic even at higher doses[10]. The various aspects of its pharmacological actions and diverse health benefits have led us to select silymarin as the drug of choice for this study.

METHODOLOGY

More than 50 different articles, published between 2000 and 2018, were studied to pool up the information on *Silybum marianum*. Silymarin was studied for its pharmacological actions on liver, the articles describing actions on silymarin on other organs are excluded from the study. The reported toxicology and drug-drug interaction was also evaluated.

DISCUSSION

Mechanism of Action: Silymarin works in various ways i.e. as an antioxidant, an anti-inflammatory agent, an anti-apoptotic agent, produces anti-fibrotic effect, influenced by controlling the permeability of

hepatocytes membrane, to forbid the entry of toxic agents. It also promotes regeneration of liver cells to heal damage. Liver is the most important homeostatic organ and the main site of detoxification of drugs. Liver damage, a major cause of death all over the world, is associated with certain environmental toxins, pharmaceutical agents, alcohol, and viral attacks[11] (Figure 3**)**.

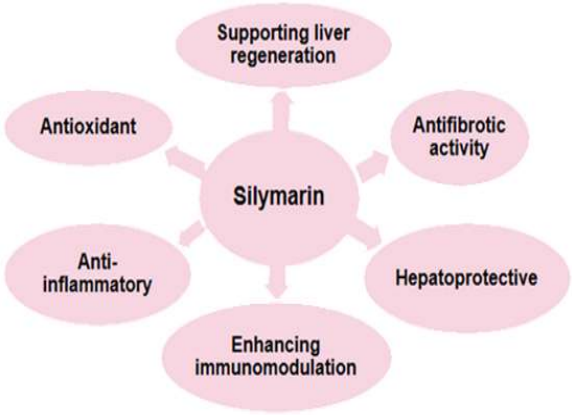

Figure 3: Actions of silymarin

Many researches have established the role of silymarin in hepato-protection. Machicao and Sonnenbichler in 1997 reported silymarin stimulates RNA polymerase to enhance rRNA production which, in turn, increases the synthesis of plasma membrane proteins to ensure its stability[11]. The antioxidant nature of silymarin contributes to its membrane stabilizing action. The interaction between flavonoid components of silymarin and polar ends (head) of phospholipids of hepatocytes' membrane prevent lipid peroxidation[12]. Liver protection is due to inhibition of synthesis of leukotriene B due to silibinin found in silymarin[13].

The anti-inflammatory action of silymarin is central in treatment of viral hepatitis and liver cirrhosis. This control of inflammation is brought by inhibiting the intrahepatic Nuclear Factor kappa B (NF-kb), which decreases Tumor Necrosis Factor-alpha (TNF-á), Interferon (IFN-g), Interleukin-2 and inducible Nitric Oxide[14]. On exposure of alcohol, carbon tetrachloride, and many other toxins, liver stellate cells rapidly turn into myofibroblasts and cause fibrogenesis, accumulation of collagen in liver. Silymarin increases level of á-SMA (smooth muscle actin) marker for detection of myofibroblasts, and acts as an anti-fibrotic agent to cure liver toxicities[15-17]. Oxidative stress is one of the prominent causes of liver impairment. Brandon Warner et al in 2010 stated silibinin as an inhibitor of Cytochrome P-450 dependent reactive oxygen species generation in liver carcinoma cells in vitro[18]. *Silybum marianum* protects gall bladder and hinders cholestasis via inhibiting cAMP-phosphodiesterase[19].

Silymarin in Liver Fibrosis and Cirrhosis: Liver injury may cause fibrosis, especially in case of chronic hepatic injuries, which in turn becomes a reason for liver cirrhosis and hepatocellular carcinoma (HCC). Liver cirrhosis alone caused approximately one million deaths worldwide in 2010[20,21].

Literature reports the cirrhosis activity by CCl_4 induced mechanism in rats. The dose of 50 mg/kg of silymarin was given orally at the last day of 4^{th}, 8^{th}, and 12^{th} weeks respectively. Histopathology of liver was assessed. Anti-fibrotic effects of silymarin were related to stimulation of liver stellate cells (through expression of TGF-â) and mast cells stabilization[22]. In a thiocetamide (100 mg/kg, intra peritoneal)-induced liver cirrhotic condition, mice at the Institute of Cancer Research (ICR) were cured with 150mg/kg silymarin ($P < 0.05$). Silymarin amended the hepatic lesions and caused down-regulation of hepatic MMP-2, MMP-13, TIMP-1, TIMP-2, AP-1, KLF6, TGF-â1, á-SMA and COL-á1[23]. To find the anti-fibrotic effects a comparative study for 8 weeks was conducted on nilotinib (10 and 20 mg/kg) and silymarin (100mg/kg). Cirrhosis was induced by CCl_4 in Wistar rats. Nilotinib 20 mg/kg reduced hepatic fibrosis by 68% and collagen formation by 49%. Silymarin decreased fibrosis and collagen formation by 47% and 18% respectively[24].

In a rat model, liver fibrosis was produced by N-nitrosodimethylamine (DMN) and oral Silibinin was induced therapeutically which reduced levels of AST, ALT, and ALP. Oxidative stress reduction and decline in collagen formation was also observed post silibinin treatment which suggests the use of silibinin in hepatic fibrosis[25]. In another study, silymarin proved its anti-fibrotic potential in vivo via changing the expression of genes associated with mitochondrial ETC (electron transport chain) and arrangement of cytoskeleton[26].

An oral dose of silymarin (20 and 100 mg/kg) was given to rats which were then given CCl_4 (2 ml/kg). Silymarin inhibited CCl_4 initiated inflammation and fibrogenesis in dose-dependent manner by reducing the gene expression of chemokine MCP-1, cytokine TGF-beta. The effect was also found on human stellate cells which was indicative of hepato-protective role of silymarin[27]. In many rat and mice models, silymarin exhibited anti-inflammatory properties in treatment of liver ailments such as cholestatic liver injury[28].

Silymarin in Non-alcoholic Fatty Liver Disease: Non-alcoholic fatty liver disease (NAFLD) is regarded

as the most common hepatic disease globally. The rate of prevalence of NAFLD is 30% in the West and majority of people develop NASH (non-alcoholic steatohepatitis) which leads to cirrhosis and hepatocellular carcinoma[29,30].

In a research, Otsuka Long-Evans Tokishima Fatty (OLETF) rats were given 390 mg/kg/day silymarin to treat NAFLD. In eight weeks, remarkable improvement occurred with reduction in symptoms of fibrosis and decline in profibrogenic elements[31,32]. In mice experiencing streptozotoin and high fat diet triggered nonalcoholic steatohepatitis (NASH), high doses of silymarin at 500-1000 mg/kg showed reduction in steatosis[33]. Four-week treatment with 20 mg/kg intra peritoneal silibinin in mice produced satisfactory cure in NASH[32]. In a 12-week open label comparative study, silymarin (70 mg three times a day) was compared with vitamin E (400 IU/day) in 71 patients, for treatment of NAFD/NASH. At the end of the study, there was significant decline in serum AST and ALT levels in both the groups. The mean AST levels returned to normal 74.6% in silymarin treated group and 56.30% in vitamin E treated group[34].

In a 24-week comparative randomized study in 2009, Hashemi et al gave 280 mg bid silymarin against placebo in 50 patients. ALT and AST levels reduced to normalization (<40) in 18% and 20% patients respectively (placebo group) and in 52% and 62% patients respectively (silymarin treated group)[35]. Few similar studies were conducted by other researchers and exhibited the efficacy of silymarin in treatment of NAFD/NASH. In two different studies (8 and 12 weeks), silymarin was detected as more effective treatment in comparison to placebo[36,37].

In a study, three different therapies i.e. 140 mg/day silymarin, pioglitazone 15 mg/day and metformin 500 mg/day were compared for the treatment of NAFD patients. Each treatment was given to 22 patients for a period of 8 weeks. The results proved that silymarin and pioglitazone are superior to metformin[38]. Another relevant study on NAFD showed that silymarin reduced release of IL-1 â, hindered the association of NLRP3 inflammatory complex, declined the accumulation of á-tubulin. MEC and Sirtuin 2 were affected by silibinin which stopped the NLRP3 inflammasome activation in mice with NASH[39]. In 2017, Wah et al evaluated the efficacy of silymarin in a double blind RCT (randomized control trial). At the doses of 700 mg thrice a day, silymarin reduced NAFLD by more than 30% as compared to placebo. Liver cirrhosis and stiffness were largely reduced[40].

Silymarin in Hepatitis: Viral hepatitis is a worldwide health issue demanding attention. Hepatitis B and C have become major causes of death. Various new approaches of treatment, which could inhibit viral replication or boost up immunity against viral hepatitis, have been introduced[41]. These treatments are not accessible to all due to their high cost. In such a scenario, silymarin, the herbal drug, is a better alternative. In 2003, Wei et al conducted a meta-analysis to determine the therapeutic efficacy of silymarin alone and in concurrence with lamivudine and interferon (anti-viral) for treating HBV chronic hepatitis. Comparable results were obtained with silymarin and the other antivirals used in diminishing aspartate aminotransferase (AST) and ALT levels. The negative conversion rate of serum HBsAg and HBeAg were found (relative risk 1.50 and 1.80 respectively) at 95% confidence interval 0.18–12.35 and 0.43–7.60 respectively[42].

Since the development of HBV vaccination in the 1980s, virus C chronic hepatitis has now become the major cause of hepatopathy[43]. In a study, Yang et al determined the effect of silibinin, given per OS and high dose of I.V. injection, on HCV-RNA serum level[44]. In another similar study, it was evaluated that I.V. administration of silibinin interferes with the lifecycle of HCV to inhibit its replication for the treatment of hepatitis C. Silibinin can inhibit RNA polymerase function in HCV[45]. Ferenci et al, studied that in patients unresponsive to peg-interferon therapy I.V. administration of silibinin, via blocking HCV polymerase enzymatic activity, at concentration of 75 ìM and 100 ìM reduced viral replication in one to four weeks[46].

A case study of a 44 years old female infected with HCV genotype-1, showed that a combination of 1200 mg/day silibinin, 1200 mg/day ribavirin, and 6000 IU/day vitamin D is effective in treatment of HCV hepatitis. The therapy was administered for 238 days[47]. In an important recent development, nano particles of silibinin have been designed. As compare to conventional drugs, these nano particles have greater bioavailability on oral administration in rodents, therefore better distribution and higher serum concentration results. In in-vitro studies, these nanoparticles diminished HCV infection in human liver cells[48]. An Egyptian researcher proved in a randomized study that silymarin in a higher dose (1050 mg/day for 12 weeks) was greater in efficacy than a usual dose of 420 mg/day for 12 weeks. The factors improved at higher doses were serum albumin level and hepatic blood flow[49].

Silymarin in Hepatocellular Carcinoma: Hepatocellular carcinoma (HCC) has become a major reason of death all over the world[50], causing approximately 0.75 million deaths every year[51]. HCC is a consequence of hepatic inflammation, viral hepatitis, NASH, and contact with toxins like alcohol[52]. The scanty availability of treatments has urged the healthcare professionals to explore herbal products for therapeutic values[53-56].

Mastron et al in 2015 proved that silymarin/silibinin reduce oxidative stress and induce arrest at various stages of cell cycle to treat hepatic cancers[57]. Bosch-Barrera et al in 2017 studied that silymarin reduced toxicity of anti-cancer drugs through inhibition of signal transducers[58]. Hepatocellular carcinoma was induced by N-nitrosodiethylamine. The treatment with silymarin considerably attenuated the changes in levels of ALT, AST, alpha fetoprotein, and decreased MDA-DNA adduct formation, hence proved itself as a likely chemotherapeutic agent[59]. In another study, N-nitrosodiethylamine (NDEA), 10 mg/kg for 12 weeks, was used to induce HCC. The parameters assessed were ALT, AST, MDA, GSH, GR, and others. The group of mice treated with silymarin showed a reduction in MDA and enhancement in GS, GR, and SOD levels. Histopathology was improved and AST and ALT were brought to normal[60]. Nano emulsion of silymarin was developed and optimized. Studies against HCC (Hepatocellular carcinoma) showed that nano emulsion enhanced ROS intensity and declined cell viability ($p<0.05$)[61].

Toxicity Studies of Silymarin: The toxicity studies, after I.V. infusion of silymarin, indicated LD_{50} of 400, 385, and 140 mg/kg in mice, rats, and rabbits respectively whereas at slow rate of I.V. infusion, LD_{50} reached 2 g/kg. After an oral dose, LD_{50} was 10g/kg[62].

As stated in 'Milk Thistle report NTP TR 565', silymarin has great safety margin[63,64]. For two years, 50 rats and mice of both genders were given 1.25%, 2.5%, and 5% of milk thistle extract. No occurrence of toxicity and carcinogenicity was observed. When determining the toxicity of silymarin in humans, no more than 2.4% and 1% occurrence of adverse effects were reported, in blinded and open clinical studies respectively. The main adverse event was a laxative effect. Other effects comprised nausea, vomiting, dyspepsia, urticaria, inflammation like pruritus and arthralgia. In all published studies of silymarin toxicity, only one serious adverse reaction is reported which led to collapse in a 57 years old female[65].

Drug-Drug Interaction Studies on Silymarin : In a 2-week study on Chinese volunteers, the administration of silymarin (140 mg tid) and talinolol (a substrate of multidrug resistant P-glycoprotein) was done concurrently. It was observed that plasma AUC of talinolol was increased by 36% by silymarin[66]. In a simulated study, the interaction of silibinin with warfarin (CYP2C9 substrate) and midazolam (CYP3A substrate) were studied. At higher dose (1,650 mg/day), silymarin was predicted to enhance midazolam and warfarin AUC approximately by 5% and 4% respectively. A clinical study suggested 9% and 13% increase in plasma AUC of midazolam and warfarin at concurrent use of high doses of silymarin[67].

CONCLUSION

The review concluded that silymarin is highly effective in treatment of various liver ailments and is free from any major toxicity. Silymarin because of its low cost, easy availability, and greater therapeutic value, has become a drug of choice. Exploration of other herbal drugs is suggested to counter the side effects of antibiotics and the emerging resistance against them.

Authors' contributions: Prof. Huma Shareef conceived the idea, edited and supervised the manuscript. Ms. Erum zaheer wrote the manuscript. Ms. Saima and Ms. Hina searched the literature. All authors discussed the results and contributed to the final manuscript.

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COMMUNICATION

Molecular Diagnostics: A Paradigm Shift

Asma Shabbir[1] and Syed Mehmood Hassan[1]

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INTRODUCTION

Effective care given to the patient by a healthcare practitioner depends upon accurate 'diagnosis' and 'treatment' of the ailment. The first line laboratory diagnostic applications like gram staining, hematoxylin and eosin staining, complete blood count, and other biochemical tests have been available for decades. However, they have significant limitations. Molecular diagnostics has offered a dynamic transformation to the field of diagnostics. It is a branch which provides a set of techniques to analyze biological markers in the genetic code (genome) and protein expressed by the genes (proteome). Its role in human diseases widely tags in mutation and pathogen detection. Continuous availability of new methods and new applications has helped molecular diagnostics to be the most rapidly growing field in the laboratory medicine.

With ground breaking developments in molecular biology, we are trying to understand the ways in which diseases develop in an individual. Moreover, it also provides us an idea of disease resistance, immunity, and specific therapy to the patient. Molecular diagnostics is widely being used in hospitals, reference laboratories, and blood banks. The major technologies involved are polymerase chain reaction (PCR), blotting techniques, fluorescent in situ hybridization (FISH), microarray, and mass spectrometry amongst others. These tests are extensively being used in clinical applications including infectious diseases, genetic disease screening, pharmacogenomics, oncology and human leukocyte antigen typing[1].

PCR is the most commonly used technique in major laboratories. It has revolutionized the field of scientific research and medicine. Approximately more than twelve modified types of PCR are now available with significant advantages over one another. It is playing an emerging role in infectious diseases like tuberculosis, HIV, hepatitis etc. Though the diagnosis of infectious agent can be done through traditional lab tests but PCR remains an exquisite choice as it is a more rapid, sensitive, and specific technique. Similarly, it has also gained importance in the field of histopathology particularly to understand the biology of lymphomas and leukaemia. Translocations in aggressive tumours like sarcomas are now being identified through RT-PCR. DNA profiling is also being utilized in forensic sciences, genetic, and paternity testing[2].

FISH technique has extended the field of cytogenetics, being more rapid and accurate as compared to the conventional method of karyotype analysis. It has become a vital tool in oncology to detect molecular markers and translocations in various cancers including leukaemia, breast carcinoma, prostate cancer, cholangiocarcinoma, and melanoma. However, literature shows that developing countries have limited use of FISH which might be due to lack of knowledge or unavailability of the technique[3]. Microarray is another upcoming technique which is not yet routinely used in diagnostics due to its cost and because it also requires skillful hands to work. But, it promises to be as reliable and sensitive as other molecular diagnostic facilities.

Other than the medical field, molecular techniques are also being utilized in agricultural and industrial applications. Moreover, research in gene therapy is ongoing and in the limelight. Approximately 19,000 to 20,000 protein coding genes are estimated to be present in a human genome[4]. In this era of personalized medicine, it is expected that complete human genome will be recognized soon through varieties of molecular techniques. Development and new insights in the field of molecular biology has provided a paradigm shift from routine diagnostic tests to the advancement in diagnosis and treatment of a number of diverse diseases.

1 Jinnah Sindh Medical University, Karachi, Pakistan

Correspondence: Dr. Asma Shabbir, Assistant Professor, Department of Pathology, Sindh Medical College, Jinnah Sindh Medical University, Karachi, Pakistan

Email: drasma52@gmail.com

CONCLUSION

Hence, we conclude that knowledge about molecular biology and diagnostics has become imperative for every medical practitioner. In this period of modern medicine, medical students as well should obtain awareness of molecular diagnostics. Moreover, focus should be on including molecular diagnostics in the medical curriculum.

Authors' contributions: Asma Shabbir conceived the idea and wrote the manuscript. Syed Mehmood Hasan did the critical review. Both the authors approved the manuscript.

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FROM THE DESK OF THE EDITOR*

3rd Pakistan International Biennial Conference on Ramadan and Health

Nazeer Khan

During recent years, there has been an emerging, upward trend in research and publication of studies on the effects of Ramadan fasting on the healthy population as well as on the populace diagnosed with various diseases and conditions. To publicize the outcomes of these research works, many awareness programs, symposia and conferences are being organized globally. International coordination groups, such as 'Diabetes and Ramadan: International Alliance' have been established to coordinate the efforts and investigations of researchers involved in this work. Periodicals like 'Journal of Nutrition, Fasting and Health of Iran' are playing a pivotal role in disseminating research outcomes related to the effects of Ramadan fasting. Guidelines for fasting are being developed for patients of various diseases, such as 'Diabetes and Ramadan: Practical Guidelines' devised by the International Diabetes Federation. 'New guidelines on diabetes management during Ramadan' has been published recently by Baqai Institute of Diabetes and Endocrinology (BIDE), Pakistan. Departments and study groups are being established in institutions to facilitate researchers in conducting studies on the effect of Ramadan fasting on different diseases.

Jinnah Sindh Medical University (JSMU), Karachi under the visionary leadership of the Honorable Vice Chancellor, Prof. Syed Muhammad Tariq Rafi, annually organizes, just before the month of Ramadan, an awareness program regarding diet and drug adjustment for conditions such as pregnancy, diabetes, renal diseases, coronary artery disease etc. Furthermore, every alternate year, JSMU organizes an 'International Biennial Conference' on Ramadan and Health. These conferences are organized to disseminate studies conducted on the effects of Ramadan fasting and these studies are presented by Pakistani speakers as well as by invited foreign speakers. The third issue of the conference, titled '3rd Pakistan International Conference on Ramadan and Health' will be held from 23rd to 25th August' 2019.

Four foreign speakers: Dr. Mohsen Nematy, Mashhad University of Medical Sciences, Mashhad, Iran; Dr. Abdolreza Norouzy, Mashhad University of Medical Sciences, Mashhad, Iran; Prof. Mafauzy Mohamed, University of Sains, Malaysia and Dr. Kamran Mahmood Ahmed A.Aziz, Ministry of Health, Abha, Saudi Arabia, have consented to present their studies in this conference. Fourteen invited speakers from Pakistan will also present their work related to various aspects of the effects of Ramadan fasting.

The theme of the conference is 'Ramadan: Bridge between Health and Spiritual Path'. The inauguration session will be held in Marriott Hotel, Karachi on the evening of 23rd August' 2019. The honourable Governor of the province of Sindh, Mr Imran Ismail and Chairman of Hilton Pharma, Dr. Sardar Muhammad Yasin Malik, have consented to be the Chief Guest and Guest of Honor, respectively, for this ceremony. Prof. Anis Ahmed, a well-known scholar and Vice Chancellor of Riphah University, Islamabad will deliver a talk on the theme of the conference. Scientific sessions would be held on the 24th and 25th of August in Sindh Medical College campus of JSMU. There will be five plenary sessions on Ramadan and Diabetes, Ramadan and Drug Management, Ramadan and Medical Education and Ramadan and Medicine; and four sessions for contributed papers. On the evening of 24th August' 2019, dinner at a beachfront restaurant along the coastline of the Arabian Sea will be hosted for the delegates and the organizing committee. The conference will culminate with closing remarks and suggestions from the delegates in the afternoon of 25th August. We cordially invite all interested delegates to come to Karachi and be part of this educational and enlightening experience. Meanwhile, we are eagerly waiting for our guests and friends to join us.

Prof. Nazeer Khan
Editor, Annals of Jinnah Sindh Medical University
Professor of Biostatistics, Chairman of the Conference,
Department of Research
Jinnah Sindh Medical University,
Rafiqui H R. Shaheed Road
Karachi 75510, Pakistan
nazeerkhan54@gmail.com
Phone: +92 21 99204776/ext 1087

* This announcement might also be published in other medical journals.

ACKNOWLEDGEMENT OF REVIEWERS

July 2018 to June 2019

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