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The newly launched peer-reviewed quarterly journal would cover full spectrum of the specialties in biomedical and clinical research. Its sixth issue would be released in July 2015. The journal aims to publish articles arising out of original research, specialized topics, review articles, editorials, and description of new diagnostic and therapeutic techniques and technologies. In addition, the journal will include pictorial reviews, letters to the editors, book review, and notices of meetings and courses. In this endeavor, the journal hopes to provide a forum for the stimulation of new developments, clinical practices and research in the field of health and allied sciences. The salient feature of the journal would be to bring out from time to time special issues focusing on specific themes of national relevance including the outcome of scientific meetings, etc. A section would be devoted exclusively to young researchers and students in order to encourage them to publish their innovative ideas and research findings. **In fact, it will be a 'student-friendly' journal.**

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Editorial

Research in medical institutions not only leads to an expansion of knowledge and discovery of new medical treatments and cures, but more importantly it also passionately blends purposeful curiosity and innovative creativity with disciplined process, patient observation, and untiring perseverance. In fact, it is the evidence-based decision making which has literally become mandatory, whether it is policy-making, improving the education system or clinical practice. In the teaching profession, a teacher through his professional knowledge, experience and effective communication skills teaches in a classroom. Critical to this process is the teacher's ability to implement new strategies which have proven effective to improve performance outcomes including: inspiring, motivating and engaging students. This cannot be accomplished unless the teacher is well informed of the recent research findings and committed to implement those in classroom. Effective teaching is also evidence-based, and eventually it is the research blended with knowledge and other teaching attributes which enables teachers to be effective in their profession, and enable institutions to serve students the best. Another important aspect of teaching is to inculcate the curiosity of innovation among students, whereby they feel interested to pursue research.

As students have varying background, skills and curiosities they may get engaged in biomedical research, patient-oriented research or research in teaching methodologies, but what is important for a teacher is to ensure that the teaching is research-oriented.

The evidence-based medicine also involves integrating clinical expertise with the best available clinical evidence derived from systematic research. In the research area, the whole spectrum of research is essential, from basic, through translational to patient-oriented research. It is essential to align the biomedical research enterprise with national needs; bringing together government, academia and industry to build upon strict principles, and that the outcomes of funding be measurable and address training, scientific consequences, technology creation, and economic benefit. Therefore, evidence-based practice has increasingly been recognized as a priority by professional organizations, influential researchers and clinicians.

At MGMIHS, research is integral to almost every realm of working, whether it is teaching, protecting the health of people or delivering community services. The management has a strong conviction that research provides new knowledge and intellectual stimulation that are vitally linked to educational process, and have reaffirmed their commitment to support research.

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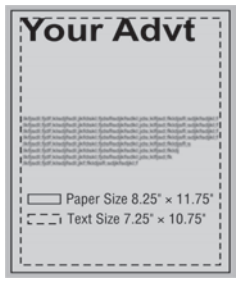

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ORIGINAL ARTICLE

Estimation of Body Fat Mass from Bioelectrical Impedance Analysis in Indian Adults Aged 23 to 81 Years: A Comparison with Dual Energy X-ray Absorptiometry

[1]BR Patil, [2]DP Patkar, [3]SA Mandlik, [4]CD Kapse, [5]GD Jindal

ABSTRACT

The purpose of this study was to validate a bioelectrical impedance analysis (BIA) equation for prediction of body fat mass (FM) against dual energy X-ray absorptiometry (DXA) in healthy Indian adults with large variations in body mass index and age. Healthy subjects (28 males and 85 females) were investigated by two methods: FM was measured by a dual energy X-ray absorptiometry and segmental bioelectrical parameters at various frequencies were measured by a commercial segmental multi-frequency BIA instrument. Total body parameters were derived from segmental bioelectrical parameters. As correlation was high and prediction error was low, a single equation was developed for FM as follows: FM = 15.45 + [0.0074 × ($R_{body250}$)] – (3.89 × sex); men = 1, women = 0) + (0.844 × w) – [6938 × (h^2/Z_{body50})] – (22.22 × h) + [3 × ($X_{body250} – X_{body5}$)/age)] + [1.53 × (Φ_{body5})] – [0.126 × (X_{body50}/h)]. Fat mass predicted with dual energy X-ray absorptiometry was 28.11 ± 9.30 kg. BIA-predicted FM was 28.12 ± 9.11 kg (R = 0.9794, adjusted R^2 = 0.9561, standard error of estimate = 1.95 kg, total error = 1.87 kg). In conclusion, the new developed BIA equation was valid for prediction of FM in healthy subjects aged 23 to 81 years with body mass indices between 15.62 and 39.98 $kg.m^{-2}$. Inclusion of reactance in the $kg.m^{-2}$ single prediction equation appeared to be essential for use of BIA equation in adults with large variations in body mass and age.

Keywords: Bioelectrical impedance analysis, Body fat mass, Body parameters, Dual energy X-ray absorptiometry.

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INTRODUCTION

Body fat percentage is the amount of body fat tissue as a percentage of total body weight. It consists of essential body fat and storage body fat. Essential body fat is necessary to maintain life and reproductive functions. Essential fat is 3 to 5% in men, and 8 to 12% in women. Storage body fat consists of fat accumulation in adipose tissue, part of which protects internal organs in the chest and abdomen. Levels of body fat are epidemiologically dependent on gender and age.[1] Different authorities have prescribed different recommendations for ideal body fat percentages. The average acceptable body fat percentages are 18 to 24% for men and 25 to 31% for women. Higher percentage of fat above average levels leads to higher health risk for weight-related illness. Assessment of fat mass (FM) in patients optimizes nutrition support to avoid or minimize muscle wasting or obesity. Therefore, nutrition assessment should include objective body-composition measurements.

A living person's exact body fat percentage generally cannot be determined, but there are several techniques which can be used to estimate it to a good degree of accuracy. Dual energy X-ray absorptiometry (DXA formerly DEXA), is a newer method for estimating body fat percentage and is a method of choice for determining body composition. There are several more complicated procedures that more accurately determine body fat percentage. Some, referred to as multicompartment models, can include DXA measurement of bone, plus independent measures of body water (using the dilution principle with isotopically labeled water) and body volume (either by water displacement or air plethysmography). Various other components may be independently measured, such as total body potassium. *In vivo* neutron activation can quantify all the elements of the body and use mathematical relations among the measured elements in the different components of the body (fat, water, protein, etc.) to develop simultaneous equations to estimate total body composition, including body fat.[2] There exist various anthropometric methods for estimating

body fat. The term anthropometric refers to measurements made of various parameters of the human body, such as circumferences of various body parts or thicknesses of skinfolds. Most of these methods are based on statistical modeling.

The bioelectrical impedance analysis (BIA) method is simple, more affordable and noninvasive way to estimate body fat percentage. In BIA, the body bioelectrical parameters (impedance, reactance, etc.) provide a measure of body fat, since the impedance to sinusoidal current varies between adipose, muscular and skeletal tissue. Fat-free mass (muscles) is a good conductor as it contains a large amount of water (approximately 73%) and electrolytes, while fat is anhydrous and a poor conductor of electric current. Many investigators have developed empirical BIA equations for prediction of body fat mass, fat free mass (FFM) and total body water (TBW).[3-13] Some of these equations have been validated in relatively young, healthy adults against several body-composition techniques.[6] Studies have shown that BIA formulae developed for healthy, normal-weight subjects are not suitable for obese subjects[14,15] and are not valid in elderly subjects.[16] In longitudinal studies, the use of different BIA formulae in the same subject who becomes overweight or older introduces a bias into body-composition studies and raises one question whether the differences in body composition are due to changes in the BIA formula or to changes in body composition. Thus, it would be advantageous to use a single formula that is applicable in young as well as elderly subjects including overweight subjects. Roubenoff et al[16] and others concluded that BIA equations are subject to errors that cannot be determined a priori unless they are validated in the specific population in which they are to be used.[17,18] Thus BIA equations must be validated in a representative population sample against a reference method before its accuracy is accepted. Bioelectrical impedance analysis can be validated against DXA, hydrodensitometry, and total body potassium. Dual energy X-ray absorptiometry is one reference method[5] that has been validated against independent methods, such as *in vivo* neutron activation,[4,8] total body potassium, and hydro densitometry.[9] It is commonly cited as the current standard for body composition testing.

To date, there has been no specific investigation about the factors that affects the accuracy and performance of BIA equation for prediction of FM in Indian adults. While using BIA method, factors that need to clarify are single *vs* multi-frequency; non-phase *vs* phase sensitive measurements; whole body *vs* segmental approaches; choice of predictor variables and subject factors. The purpose of this study was to validate a single BIA equation for prediction of body FM against DXA in healthy Indian adults with large variations in body mass index and age.

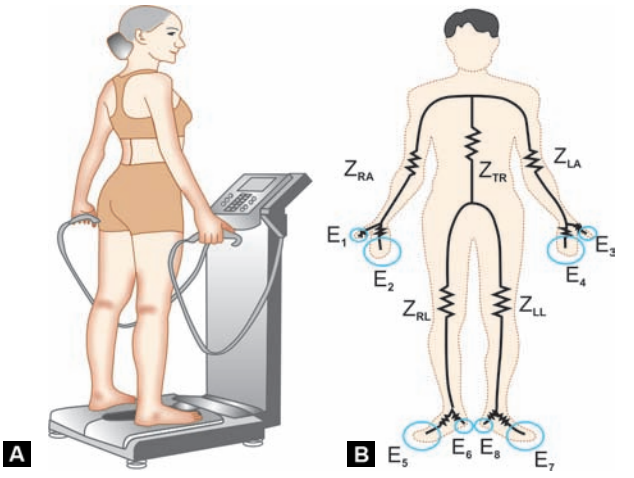
SUBJECTS AND METHODS

Subjects

The study group consisted of 113 subjects (28 males and 85 females) in the age group of 23 to 81 years. All subjects were born in India and resided in Mumbai. The subjects were apparently healthy and none was under medication during the last 1 week. The subjects were kept fasting for nearly 2 hours before the measurements. The standing height of each subject was measured to the nearest 0.5 cm. The purpose of the study was explained to all the subjects and their oral consent was taken. Each subject was measured by two methods: FM was measured by dual energy X-ray absorptiometry (Lunar Prodigy, DPX-IQ, General Electric Healthcare, Belgium, Europe) and the segmental bioelectrical parameters (impedance and reactance) at various frequencies were measured by a commercial segmental multi-frequency BIA instrument InBody720 (Biospace Co. Ltd. Seoul, Korea). The total body parameters were derived from segmental parameters.

Calculation of Total Body Bioelectrical Parameters

InBody 720 provides the segmental bioelectrical parameters (impedance and reactance) at various frequencies using eight-polar tactile-electrode. This commercial product is approved by FDA (Food and Drug Administration, United States, May 2003). It is based on the segmental impedance measurement approach and operated at frequencies of 1, 5, 50, 250, 500 and 1000 kHz, which are pre-set by the manufacturer and introduced into the body in the ascending order of frequency. This device uses contact electrodes located in the handgrips and the footpads. Subjects were asked to stand with the ball and heel of each foot on two metallic electrodes on the floor scale and hold handrails with metallic grip electrodes in contact with the palm and thumb. Laboratory temperature was maintained at 25 ± 2°C, in order to avoid any variation in electrical impedance due to temperatures. They were instructed to keep their arms fully extended and abducted approximately 20° laterally (Figs 1A and B). The sequence of measurements were controlled by a microprocessor, proceeds as follows. For 1 kHz programed frequency, an alternating current of 100 μA and for other programed frequencies, an alternating current of 500 μA of intensity (I) is applied between E1 and E5. The recorded voltage difference (V) between E2 and E4 is divided by I to obtain the impedance of the right arm (Z_{RA}). The same operation is performed with V recorded between E4 and E8 to obtain the trunk impedance (Z_{TR}) and with V recorded between E6 and E8 to obtain the impedance of the right leg (Z_{RL}). The alternating current is then applied between E3 and E7



Figs 1A and B: Subject position on InBody 720 (A) for measurement of segmental bioelectrical parameters. The eight electrode positions (B) on the body

and the value of V measured between E2 and E4 is used to calculate the impedance of the left arm (Z_{LA}). Lastly, the value of V measured between E6 and E8 is used to calculate the impedance of the left leg (Z_{LL}). No precaution was taken to standardize the subject's posture before BIA, as suggested by the manufacturer. The instrument gave the impedance values of five segments (e.g. arms, trunk and legs) from the measurements at 6 frequencies (1, 5, 50, 250, 500, 1000 kHz) and also the reactance values at three frequencies (5, 50, 250 kHz).

From segmental impedance and reactance values, the respective values of segmental resistance and phase angle were calculated at three frequencies (5, 50, 250 kHz) using the following equations:

Resistance of a segment at 'f' kHz frequency,

$$R_{sf} = \sqrt{(Z_{sf})^2 - (X_{sf})^2} \quad (1)$$

Phase angle of a segment at 'f' kHz frequency,

$$\phi_{sf} = \tan^{-1}\left(\frac{X_{sf}}{R_{sf}}\right) \quad (2)$$

Where, $[(R)_{sf}]$ is the resistance of the body segment, (Z_{sf}) is the impedance of the body segment, $[(X)_{sf}]$ is the reactance of the body segment and $[(\phi)_{sf}]$ is the phase angle of the body segment at 'f' kHz frequency.

Values of resistance, reactance, impedance and phase angle of total body at three frequencies (5, 50 and 250 kHz) were calculated as follows:

Body resistance at 'f' kHz frequency,

$$R_{bodyf} = (R_{RAf}) + (R_{LAf}) + (R_{TRf}) + (R_{RLf}) + (R_{LLf}) \quad (3)$$

Body reactance at 'f' kHz frequency,

$$X_{bodyf} = (X_{RAf}) + (X_{LAf}) + (X_{TRf}) + (X_{RLf}) + (X_{LLf}) \quad (4)$$

Body impedance at 'f' kHz frequency,

$$[Z_{bodyf}] = \sqrt{[R_{bodyf}]^2 + [X_{bodyf}]^2} \quad (5)$$

Phase angle of body at 'f' kHz frequency,

$$[\phi_{bodyf}] = \tan^{-1}\frac{[X_{bodyf}]}{[R_{bodyf}]} \quad (6)$$

where $[R_{bodyf}]$ is the resistance of the body, $[Z_{bodyf}]$ is the impedance of the body, $[X_{bodyf}]$ is the reactance of the body and $[\phi_{bodyf}]$ is the phase angle of the body at 'f ' kHz frequency. The body segments right arm, left arm, trunk, right leg and left leg are represented by RA, LA, TR, RL and LL respectively.

Dual Energy X-ray Absorptiometry

Fat mass of each subject was measured by DXA. The DXA scanning technique measures the differential attenuation of two different levels of X-ray energy as they pass through the body, thereby allowing determination of bone mineral content and soft-tissue mass on a pixel-by-pixel basis. Dual energy X-ray absorptiometry allows the separation of total and segmental body mass (BM) into fat mass (FM), lean tissue mass (LTM) and bone mineral content (BMC). The sum of LTM and BMC gives fat-free mass (FFM). The X-ray source (fan beam) mounted beneath the patient generates a narrow, tightly collimated beam of X-ray that pass through the patient at rapidly changing energies. The transmitted intensity of each energy level is measured by a radiation detector mounted on a movable arm directly above the X-ray source. Simultaneous with the measurement of the skeleton, the percentage of fat is determined from the attenuation ratio of lower energy to higher energy detected by the beam. This ratio is calculated from all non-skeleton pixels scanned and extrapolated of the skeleton-containing pixels. In the literature,[28] the effective total body-radiation dose was reported as 5.4 μSv. All measurements were performed with a Lunar Prodigy instrument DPX-IQ, General Electric Healthcare, Belgium, Europe (Fig. 2).

Statistics

The total body bioelectrical parameters like (Z_{body}), (X_{body}), (Φ_{body}) and (R_{body}) are derived from segmental parameters at 5, 50 and 250 kHz frequencies. The physical parameters comprise of weight (w), stature (h), gender and age. By combining physical and bioelectrical parameters, the new parameters like (h^2/Z_{body}), (Z_{body}/w), (Z_{body}/h), (R_{body}/h), (X_{body}/h), $(w \times h^2/Z_{body})$, etc. are derived at 5, 50 and 250 kHz frequencies. Descriptive statistics for all parameters were calculated and expressed as mean

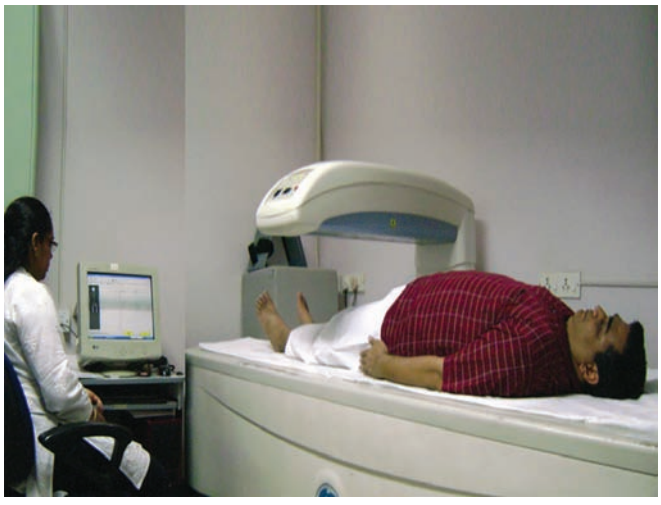


Fig. 2: Measurement of fat mass by dual-energy X-ray absorptiometry (lunar prodigy, DPX-IQ and general electric healthcare)

± standard deviation (SD). Simple regressions were calculated to test correlations between FM obtained from DXA and BIA parameters. FM measured by DXA was used as the criterion measurement.

With the help of SPSS package version 17, stepwise multiple regression analysis was carried out to derive BIA prediction equation for FM. The predictor variables entered into the BIA model in the order of highest correlation coefficient and smallest standard error of estimation (SEE) were ($R_{body250}$), (sex), w, (h^2/Z_{body50}), h, ($X_{body250} - X_{body5}$)/age, (Φ_{body5}), (X_{body50}/h). In addition to correlation and regression techniques, error analysis was performed. Standard error of estimation was calculated and used as error of prediction for DXA derived FM and predicted FM by new BIA equation. The total error (TE) was calculated as:

$$TE = \sqrt{\frac{\sum_{i=1}^{n} [(FM1)i - (FM2)i]^2}{n}} \quad (7)$$

Where (FM1) is the observed value of FM by DXA and (FM2) is the predicted value of FM by BIA equation. The total error of measurement estimates the magnitude of the error for a given measurement and is defined as the difference between measurements for the individual (i), i = 1… n, where n is the number of individuals.[20] The total error was compared with the SEE. A small difference between total error and SEE indicates high accuracy of the prediction. To assess the agreement between the two clinical measurements, the difference between the values was plotted against their means, because the mean was the best available estimate of the true value. This analysis allows for the calculation of bias (estimated by the mean differences), the 95% confidence interval for the bias and the limits of agreement (two SDs of the difference).[19] Statistical significance was set at $p \leq 0.05$ for all tests. In order to verify the different factors which may affect the performance of the BIA method, the different sets of parameters were selected and for each set, with the help of SPSS package version 17, stepwise multiple regression analysis was carried out to derive BIA prediction equation for FM. The change in % adjusted R^2 and SEE were recorded for each set of variables.

RESULTS

A total of 113 healthy adults aged 23 to 81 years and weighting 39 to 97.7 kg were used as subject. Table 1 shows the anthropometric and BIA characteristics of male and female subjects grouped by age. The prediction equation developed from all subjects is shown in Table 2. The order of entry of predictor variables was ($R_{body250}$), (sex), w, (h^2/Z_{body50}), h, (($X_{body250} - X_{body5}$)/age)), (Φ_{body5}), (X_{body50}/h). For the BIA model (Table 3) weight (w) alone accounted for 64.26% of the variability (SEE = 5.56 kg) of the equation whereas (X_{body50}/h) accounted for 13.75% of the variability (SEE = 8.63 kg). Inclusion of w, (age), h and (sex) without BIA parameters accounted for 92.73% of the variability with a SEE of 2.51 kg. Thus inclusion of BIA parameters clearly improved the prediction power (95.61%) and decreased the SEE (1.95 kg) compared with anthropometric parameters only. The significant variables (predictors) for estimating the value of FM are listed in Table 4. The beta (standardized regression coefficient) value is the measure of how strongly each predictor variable influences the criterion variable. Higher the absolute beta value, greater is the impact of the predictor variable on criterion variable. The t-value and statistical significance (p) value give the rough indication of the impact of each predictor variable: a big absolute t-value and small p-value suggests that a predictor variable has a large impact on the criterion variable. The tolerance values are a measure of the correlation between the predictor variables and can vary between 0 and 1. The closer to zero the tolerance value is for a variable, the stronger the relationship between this and the other predictor variables. We should worry about the variables that have a very low tolerance. Variance inflation factor is an alternative measure of collinearity (in fact it is the reciprocal of tolerance) in which a large value indicates a strange relationship between the predictor variables. Graph 1A shows a comparison of FM measured by BIA equation (a new non-invasive technique) and DXA (established method). Graph 1B shows the correlation and mean difference, according to Bland and Altman,[19] using the prediction equation in all subjects. Here, the mean difference was 0.018 kg; the 95% confidence interval was from –3.737 to

Table 1: Anthropometric and bioelectrical impedance analysis characteristics of healthy subjects grouped by age

	Age (years)			
	Below 40	*41–50*	*51–60*	*Above 60*
Male (n = 28)				
n	7	7	9	5
h (m)	1.67 ± 0.076	1.71 ± 0.101	1.70 ± 0.056	1.70 ± 0.033
w (kg)	70.56 ± 14.15	76.36 ± 3.48	73.51 ± 12.30	71.30 ± 6.01
BMI (kgm^{-2})	25.01 ± 3.56	26.38 ± 2.13	25.37 ± 3.71	24.64 ± 1.93
(Z_{body5}) (Ω)	1333.28 ± 161.34	1272.47 ± 84.30	1288.99 ± 165.05	1323.97 ± 121.87
(Z_{body50}) (Ω)	1167.17 ± 137.35	1112.27 ± 86.41	1153.75 ± 153.52	1210.24 ± 151.46
($Z_{body250}$) (Ω)	1055.77 ± 119.43	1007.59 ± 79.17	1049.99 ± 140.68	1086.27 ± 113.85
(R_{body5}) (Ω)	1331.52 ± 161.21	1270.96 ± 84.46	1287.75 ± 165.06	1322.62 ± 121.88
(R_{body50}) (Ω)	1161.14 ± 136.21	1116.56 ± 86.42	1149.16 ± 153.18	1205.78 ± 151.48
($R_{body250}$) (Ω)	1052.19 ± 118.84	1003.82 ± 78.66	1046.81 ± 140.20	1083.13 ± 113.66
(X_{body5}) (Ω)	68.43 ± 7.77	61.13 ± 9.84	56.00 ± 8.53	59.20 ± 7.95
(X_{body50}) (Ω)	118 ± 20.91	112.44 ± 12.69	102.53 ± 12.84	103.26 ± 11.12
($X_{body250}$) (Ω)	85.96 ± 13.40	86.15 ± 15.94	81.59 ± 12.92	82.48 ± 8.59
(Φ_{body5}) (degree)	2.948 ± 0.185	2.767 ± 0.499	2.508 ± 0.389	2.573 ± 0.357
(Φ_{body50}) (degree)	5.779 ± 0.570	5.766 ± 0.639	5.116 ± 0.394	4.928 ± 0.556
($\Phi_{body250}$) (degree)	4.658 ± 0.343	4.898 ± 0.731	4.452 ± 0.311	4.364 ± 0.287
(h^2/Z_{body5}) ($m^2\ \Omega^{-1}$)	0.00214 ± 0.00037	0.00229 ± 0.00020	0.00228 ± 0.00033	0.00220 ± 0.00025
(h^2/Z_{body50}) ($m^2\ \Omega^{-1}$)	0.00224 ± 0.00041	0.00259 ± 0.00022	0.00255 ± 0.00039	0.00243 ± 0.00035
($h^2/Z_{body250}$) ($m^2\ \Omega^{-1}$)	0.00269 ± 0.00044	0.00289 ± 0.00023	0.00280 ± 0.00043	0.00269 ± 0.00036
Female (n = 85)				
n	11	22	22	30
h (m)	1.57 ± 0.028	1.58 ± 0.058	1.57 ± 0.049	1.55 ± 0.052
w (kg)	64.96 ± 17.71	63.87 ± 12.09	68.24 ± 11.28	64.46 ± 15.32
BMI (kgm^{-2})	26.29 ± 7.28	25.55 ± 5.24	27.74 ± 4.24	26..88 ± 5.79
(Z_{body5}) (Ω)	1584.09 ± 258.92	1600.89 ± 205.91	1522.76 ± 190.49	1563.15 ± 232.68
(Z_{body50}) (Ω)	1419.39 ± 224.65	1449.84 ± 195.48	1380.75 ± 172.56	1433.10 ± 217.48
($Z_{body250}$) (Ω)	1288.10 ± 199.54	1321.71 ± 189.54	1266.63 ± 161.01	1320.25 ± 203.69
(R_{body5}) (Ω)	1582.59 ± 258.58	1599.60 ± 205.87	1521.55 ± 190.32	1562.12 ± 232.54
(R_{body50}) (Ω)	1413.58 ± 223.41	1444.95 ± 195.31	1376.31 ± 172.15	1429.11 ± 217.01
($R_{body250}$) (Ω)	1283.91 ± 198.46	1317.90 ± 189.14	1263.09 ± 160.55	1316.77 ± 203.18
(X_{body5}) (Ω)	68.53 ± 15.45	63.56 ± 10.90	60.07 ± 12.20	55.65 ± 14.69
(X_{body50}) (Ω)	127.95 ± 25.51	118.55 ± 13.04	109.90 ± 16.92	105.89 ± 20.46
($X_{body250}$) (Ω)	103.63 ± 22.01	100.14 ± 13.83	94.36 ± 14.45	95.38 ± 17.218
(Φ_{body5}) (degree)	2.473 ± 0.275	2.291 ± 0.371	2.259 ± 0.349	2.027 ± 0.499
(Φ_{body50}) (degree)	5.159 ± 0.387	4.722 ± 0.419	4.576 ± 0.503	4.248 ± 0.589
($\Phi_{body250}$) (degree)	4.585 ± 0.319	4.358 ± 0.269	4.275 ± 0.364	4.146 ± 0.414
(h^2/Z_{body5}) ($m^2\Omega^{-1}$)	0.00159 ± 0.00026	0.00159 ± 0.00020	0.00164 ± 0.00023	0.00156 ± 0.00028
(h^2/Z_{body50}) ($m^2\Omega^{-1}$)	0.00178 ± 0.00029	0.00176 ± 0.00024	0.00181 ± 0.00025	0.00171 ± 0.00031
($h^2/Z_{body250}$) ($m^2\ \Omega^{-1}$)	0.00196 ± 0.00031	0.00194 ± 0.00028	0.00197 ± 0.00028	0.00186 ± 0.00034

n: number of subjects; h: height; w: weight; BMI: body mass index; Z_{body5}: body impedance at 5 kHz; Z_{body50}: body impedance at 50 kHz; $Z_{body250}$: body impedance at 250 kHz; R_{body5}: body resistance at 5 kHz; R_{body50}: body resistance at 50 kHz; $R_{body250}$: body resistance at 250 kHz; X_{body5}: body reactance at 5 kHz; X_{body50}: body reactance at 50 kHz; $X_{body250}$: body reactance at 250 kHz; Φ_{body5}: body phase angle at 5 kHz; Φ_{body50}: body phase angle at 50 kHz; $\Phi_{body250}$: body phase angle at 250 kHz

3.773 kg. The mean difference is closer to the zero, i.e. the bias is negligible. Thus, BIA equation tends to give a closer reading having the limits of agreement (–3.737 to 3.773 kg) are small enough for us to be confident that BIA equation can be used for assessment of FM.

The elderly subjects were analyzed separately to determine whether greater error occurred with the BIA equation in aged subjects (Table 5). Fat mass measured for 35 subjects with age above 60 years from DXA was 28.9222 ± 10.1149 kg. Fat mass predicted by equation was 28.6267 ± 9.8814 kg. The mean difference was 0.2955 ± 1.5354 kg ($p > 0.05$, paired t-test; R = 0.9885, total error = 1.7481 kg). Subjects with BMIs above 27 $kg.m^{-2}$ were also analyzed separately to determine whether greater error occurred with the BIA equation in obese subjects (Table 6). Fat mass measured for 44 subjects with BMIs above 27 kgm^{-2}

Table 2: Bioelectrical impedance analysis prediction equation for fat mass using all subjects (n = 113)

FM = 15.45 + (0.0074($R_{body250}$)) – (3.89 × (sex); men = 1, women = 0) + (0.844 w) – (6938 × (h^2/Z_{body50})) – (22.22 × h) + (3 × (($X_{body250}$ – X_{body5})/age)) + (1.53 × (Φ_{body5})) – (0.126 × (X_{body50}/h))
FM predicted with DXA = 28.11 ± 9.30 kg
FM predicted with BIA equation = 28.12 ± 9.11 kg (R = 0.9794, adjusted R^2 = 0.9561, SEE = 1.95 kg, TE = 1.87 kg)

n: number of subjects; FM: fat mass; DXA: dual energy X-ray absorptiometry; BIA: bioelectrical impedance analysis; w: weight; h: height; $R_{body250}$: body resistance at 250 kHz; Z_{body50}: body impedance at 50 kHz; X_{body5}: body reactance at 5 kHz; X_{body50}: body reactance at 50 kHz; $X_{body250}$: body reactance at 250 kHz; Φ_{body5}: Phase angle of the body at 5 kHz; R: validity coefficient; SEE: standard error of the estimate; TE: total error

Table 3: Contribution and order of entry of variables to the bioelectrical impedance analysis model and anthropometric model for fat mass (n = 113 subjects)

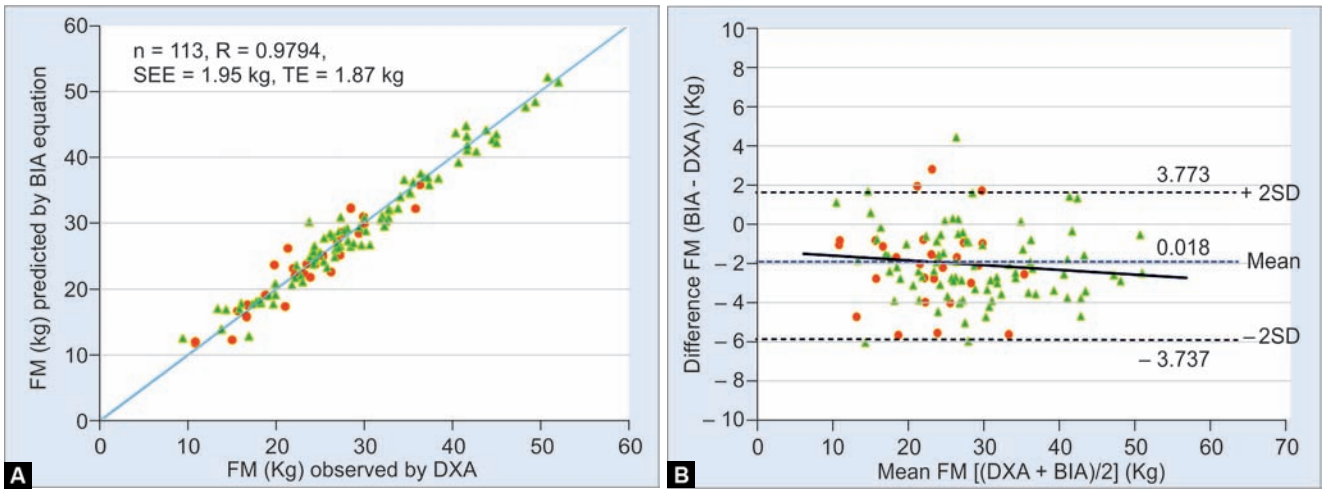
	Cumulative variables used in model			*Variables*		
Model and variables	*% Ad.R^2*	*SEE*	*p*	*% Ad.R^2*	*SEE*	*p*
BIA						
($R_{body250}$)	13.65	8.64	0.000	13.65	8.64	0.000
+ (sex)	49.55	6.60	0.000	08.08	8.91	0.001
+ w	91.97	2.68	0.000	64.26	5.56	0.000
+ (h^2/Z_{body50})	94.78	2.12	0.000	02.43	9.18	0.054
+ h	95.00	2.08	0.020	00.14	9.29	0.248
+ [($X_{body250}$ – X_{body5})/age)]	95.06	2.07	0.133	06.67	8.98	0.003
+ (Φ_{body5})	95.09	2.06	0.198	00.00	9.33	0.689
+ (X_{body50}/h)	95.61	1.95	0.000	13.75	8.63	0.000
BMI						
w				64.26	5.56	0.000
w + (age)				65.19	5.48	0.049
w + (age) + h				84.21	3.69	0.000
w + (age) + h + (sex)				92.73	2.51	0.000

n: number of subjects; BIA: bioelectrical impedance analysis; BMI: body mass index; h: height; w: weight; $R_{body250}$: body resistance at 250 kHz; Z_{body50}: body impedance at 50 kHz; X_{body5}: body reactance at 5 kHz; X_{body50}: body reactance at 50 kHz; $X_{body250}$: body reactance at 250 kHz; Φ_{body5}: phase angle of the body at 5 kHz; R: validity coefficient; SEE: standard error of the estimate; TE: total error; p: significance of contribution of each additional individual parameter to the stepwise multiple regression model; %Ad.R^2: percentage adjusted squared value of the validity coefficient

Table 4: Significant variables in bioelectrical impedance analysis model

	Standardized coefficient (beta)	*t-value*	*p*	*Collinearity statistics*	
				Tolerance	*VIF*
($R_{body250}$)	0.163	1.887	0.062	0.053	18.940
(sex)	–0.181	–4.657	0.000	0.258	3.871
w	1.212	32.615	0.000	0.284	3.522
(h^2/Z_{body50})	–0.327	–2.805	0.006	0.029	34.587
h	–0.191	–3.028	0.003	0.098	10.190
[($X_{body250}$ – X_{body5})/age]	0.106	3.194	0.002	0.356	2.808
(Φ_{body5})	0.077	2.018	0.046	0.271	3.696
(X_{body50}/h)	–0.169	–3.656	0.000	0.184	5.429

h: height; w: weight; $R_{body250}$: body resistance at 250 kHz; Z_{body50}: body impedance at 50 kHz; X_{body5}: body reactance at 5 kHz; X_{body50}: body reactance at 50 kHz; $X_{body250}$: body reactance at 250 kHz; Φ_{body5}: phase angle of the body at 5 kHz; p: significance of contribution of each additional individual parameter to the stepwise multiple regression model



Graphs 1A and B: (A) Correlations of fat mass (FM) in all subjects measured by DXA and predicted by BIA equation, (B) differences of FM in all subjects determined by using BIA and DXA. Solid line represents the linear relation between mean differences in FM (FM by BIA-FM by DXA) and average FM ([FM by DXA + FM by BIA]/2); dark abbreviated line represents the mean difference and light abbreviated lines represent the 95% confidence interval. BIA, bioelectrical impedance analysis; DXA, dual energy X-ray absorptiometry; n: number of subject; R: validity coefficient; SEE: standard error of estimation; TE: total error; circles indicate men; triangles indicate women

Table 5: Paired t-test for FM by DXA Vs BIA predicted FM for subjects with age above 60 years (n = 35)

	Mean ± SD
FM (kg) by DXA	28.9222 ± 10.1149
BIA predicted FM (Kg)	28.6267 ± 9.8814
Difference (FM by DXA-BIA predicted FM) (Kg)	0.2955 ± 1.5354
95% confidence interval for mean difference: (–4.48, 5.07)	
t-test of mean difference = 0 (Vs not = 0); t-value = 0.12; p-value = 0.902; DF = 67	

n: number of subjects; FM: fat mass; BIA: bioelectrical impedance analysis; DXA: dual energy X-ray absorptiometry; SD: standard deviation; p: level of significance; DF: degree of freedom

Table 6: Paired t-test for FM by DXA Vs BIA predicted FM for subjects with BMI above 27 kgm^{-2} (n = 44)

	Mean ± SD
FM (kg) by DXA	36.5356 ± 7.6410
BIA predicted FM (Kg)	36.4505 ± 7.5671
Difference (FM by DXA-BIA predicted FM) (Kg)	0.0851 ± 1.9586
95% confidence interval for mean difference: (–3.14, 3.31)	
t-test of mean difference = 0 (Vs not = 0); t-value = 0.05; p-value = 0.958; DF = 85	

n: number of subjects; FM: fat mass; BIA: bioelectrical impedance analysis; DXA: dual energy X-ray absorptiometry; SD: standard deviation; p: level of significance; DF: degree of freedom

from DXA was 36.5356 ± 7.6410 kg. Fat mass predicted by equation was 36.4505 ± 7.5671 kg. The mean difference was 0.0851 ± 1.9586 kg ($p > 0.05$, paired t-test; R = 0.9669, total error = 0.5643 kg). Thus, it is possible to estimate FM with the same equation for elderly and obese subjects.

DISCUSSION

Validity of BIA

It is generally agreed that the accuracy of BIA depends on the variables included in the prediction equation and on using a specific prediction equation validated for a specific population.[17] Our equation for BIA-predicted FM was used, in order of entry, ($R_{body250}$), (sex), w, (h^2/Z_{body50}), h, [($X_{body250} - X_{body5}$)/age], (Φ_{body5}), (X_{body50}/h). Although weight (w) alone accounted for 64.26% of the variability (Table 3), all other variables entered added significantly to the BIA-predicted FM. The correlation obtained with the new developed equation is observed to be 97.94% against DXA. The cross validation of BIA equation is important to test for its accuracy. Slightly lower accuracy could be expected in subjects older than 81 years. Validity of the BIA equation in subjects older than 81 years is unknown and requires further validation; it is also necessary in subjects with BMIs below 15.62 kgm^{-2}. In addition, validation studies must be conducted in subjects with nutritional disorders that affect body water. Further validation of BIA is necessary to understand the mechanisms for the changes in acute illness, fat/lean mass ratios, extreme heights, and body shape abnormalities.

Variations in BIA Parameters

Measurement of body parameters by segmental multiple frequency bioelectrical impedance analysis (MF-BIA) technique with tetra-polar electrode gives high degree of accuracy and precision. Bedogni et al[21] found that the precision of eight-polar segmental MF-BIA technique was quite good (coefficient of variation, CV < 3.0% for between days; CV < 2% for within days measurements) as compared to four-polar total body BIA performed at 50 kHz.

Several factors are known to influence the bioelectrical parameters, such as age, height, weight, gender and ethnic origin.[22,23] Several investigators have found a positive relation between age and impedance[24,25] a negative relation between age and reactance,[24-26] and a negative relation between age and phase angle.[25-27] The present study (Table 1) too, validated that a negative relation between age and phase angle in both genders. Impedance and resistance increased with age in both sexes after 60 years. Reactance decreased with age progressively in men and women until 60 years. Bioelectrical impedance analysis parameters are frequency dependent. For any individual, body impedance and resistance decrease as frequency increases. However, the body reactance and the phase angle initially increase with frequency but after certain frequency they decrease with the increase in frequency (Table 1).

Bioelectrical impedance analysis values are affected by numerous variables including body position, hydration status, consumption of food and beverages, ambient air, skin temperature, recent physical activity, and conductance of the examining table. Reliable BIA requires standardization and control of these variables. A specific and well-defined procedure for performing routine BIA measurements is required.

Factors affecting the Performance of BIA Equation

In order to verify the different factors which may affect the performance of the BIA equation, the different sets of parameters were selected and for each set, with the help of SPSS package version 17, stepwise multiple regression analysis was carried out to derive BIA prediction equation for FM. The change in '% adjusted R^{2}' and SEE were recorded for different set of variables. Through this exercise, it was found that simple measurement at 50 kHz was not sufficient. By adding frequencies help in the improvement of prediction and reducing the error. The single frequency measurement results at 50 kHz are superior to the results at 5 kHz or 250 kHz. It was also found that, inclusion of reactance/phase angle improves the prediction and reduces the error.

CONCLUSION

The results of this study show that the newly developed single prediction BIA equation validated against DXA can be used to predict FM in subjects aged 23 to 81 years and with BMIs ranging from 15.62 to 39.98 kgm^{-2}. The BIA equation developed can be used in populations with large variations in age and body mass.

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ORIGINAL ARTICLE

Causes of Domestic Violence in Married Women with Psychotic and Non-psychotic Illness

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ABSTRACT

Background: Women are integral to all aspects of society. They are worshipped, but when it comes to dealing with them, much still remains. Women bear the burden of responsibility associated with being wives, mothers and carers of others. There is a dearth of case-control studies. Domestic violence in women with psychiatric morbidity has not received sufficient attention. Domestic violence can often lead to victims developing mental health problems, and people with mental health problems are more likely to experience domestic violence. People diagnosed with mental illness are more likely than others to be victims of domestic violence. Psychiatric morbidity as a determinant of domestic violence has received little attention. Indian culture is unique and there is limited work on domestic violence from Eastern Uttar Pradesh.

Objective: To assess the magnitude and compare the cause of domestic violence in married women with psychotic and non-psychotic illness.

Materials and methods: Sixty-five women attending psychiatry outpatient department (OPD) of SSL Hospital with 35 women with psychotic illness and 30 non-psychotic illness were studied for the magnitude of domestic violence by their husband. Domestic violence questionnaire was used. Women diagnosed as suffering from Axis–I disorder as per DSM IV TR.

Results: Significantly more women in psychotic illness than non-psychotic illness reported domestic violence (total/ psychological and physical) by their husbands in past year (women with psychotic illness: 80% total/psychological violence; 65.7% physical violence and non-psychotic illness: 50% total/ psychological violence; 43.3% physical violence). Total domestic violence with psychiatric morbidity was observed in 66.2%.

Conclusion: Women with psychotic illness have a higher reporting of domestic violence by their husbands during the past years. Women with mental disorders are likely to be victims of violence. Mental disorder may increase vulnerability to domestic violence by increasing the likelihood of women being in unsafe relationships and environments and increase their vulnerability to violent victimization.

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INTRODUCTION

Violence against women is a social and public health problem. Its impact on the physical and mental health of women and their social functioning is pernicious.[3] A growing body of research confirms the prevalence of physical violence in all parts of the globe. As per the World Health Organization (WHO) multi-country study involving 10 countries, the proportion of ever-partnered women who ever experienced physical or sexual violence, or both, by their partners in their lifetime, ranged from 15 to 71% of women, with most sites falling between 29 and 62%.[16] 65.8% had identified a domestic violence victim at least once in the past 1 year.[5] Estimates of domestic violence within India vary widely from 18 to 70%.

No country or community is untouched by violence. Each year, more than 1.6 million people worldwide lose their lives to violence. For every one who dies as a result of violence, many more are injured and suffer from a range of physical, sexual, reproductive and mental health problems.[7]

Psychiatric symptoms in women are common and result in distress and varying degrees of disability. The latter may adversely affect women's sexual behavior and ability to carry out the domestic chores.

In a recent study comprising four states from Eastern India, *viz*. Bihar, Jharkhand, Odisha and West Bengal, age, education, occupation, marital duration and husband's alcoholism emerged as significant predictors of victimization and perpetration of all types of domestic violence.[2] A higher level of family income was found to be highly protective against the risk of violence. In another study conducted on rural community of Northern India revealed that an alcoholic husband emerged as the main cause for domestic violence.[8]

The protection of women against Domestic Violence Act 2005 recognizes four types of domestic violence, such

as physical abuse, sexual abuse, verbal and emotional or economic abuse.[15] For the purposes of this Act, any act, omission or commission or conduct of the respondent shall constitute domestic violence in case it:

- Harms or injures or endangers the health, safety, life, limb or well-being, whether mental or physical, of the aggrieved person or tends to do so and includes causing physical abuse, sexual abuse, verbal and emotional abuse and economic abuse; or
- Harasses, harms, injures or endangers the aggrieved person with a view to coerce her or any other person related to her to meet any unlawful demand for any dowry or other property or valuable security; or
- Has the effect of threatening the aggrieved person or any person related to her by any conduct mentioned in clause (a) or clause (b); or
- Otherwise injures or causes harm, whether physical or mental, to the aggrieved person.

Violence affects all aspects of conversational, behavioral and dynamic of individual who are exposed to violence.[12] Domestic violence is when one partner in an intimate relationship abuses the other.

Most studies on domestic violence have been population-based. The relationship between domestic violence and psychiatric morbidity has not been sufficiently explored. Twenty-one percent of domestic violence in women who attended an outpatient clinic in a North of England Hospital was reported. Women who were subjected to domestic violence tended to have more consultations and were more likely to complain of certain symptoms.[6]

There is limited data from developing countries regarding the link between domestic violence and psychiatric morbidity. Links between domestic violence and sexually transmitted diseases have been reported.[10]

Domestic spousal violence against women in developing countries like India is now beginning to be recognized as a widespread health problem impeding development. However, there is limited work in this area. There is a dearth of research tools for assessing the magnitude and pattern of domestic violence.

The causes of domestic violence in the women with psychotic illness and non-psychotic illness have not been studied well in the Indian population especially in Northern India.

MATERIALS AND METHODS

This was a descriptive study, using a quantitative approach performed. The sample comprised of 35 women with psychotic illness and 30 women with non-psychotic illness at a selected from psychiatry department of OPD and ward of Sir Sunder Lal Hospital, Banaras Hindu University, Varanasi, Uttar Pradesh, India over a period of 3 months. Inclusion criteria for the present study includes: (1) Age group between 16 and 40 years; (2) subjects who were ready to participate for the interview; (3) all the participant were attending the psychiatry OPD/Ward of SSH, BHU; (4) married female. The structured questionnaire was used and sample size was 65, where the data was collected through face to face interview, after taking written informed consent. The subjects were given a brief introduction of the purpose of the study. The study protocol was approves by the institutional ethical committee. The study sample was assessed using the following instruments:

- Sociodemographic performa includes: age, education status, type of family, occupational status. Socio-economic scale (SES) of Kuppuswamy was used for assessing SES.[9,11]
- Domestic violence questionnaire[4]: The objective of this questionnaire is to know whether there are such experiences in your marital life of mental illness women. The domestic violence questionnaire (DVQ) comprises a set of 20 questions which enquires about the frequency of domestic violence in the past. It was intended to be a short, simple, self-administered, discriminative instrument. It was designed with the intention of capturing the major dimensions of the concept of psychological and physical violence. It has been standardized on the Indian population. The total frequency is noted and scoring is done over the past 1 year. The reliability of the tool was confirmed by using Karl Pearson correlation coefficient formula and spearman's brown prophecy formula that obtained 'r'= 0.86, which showed that the tool was reliable.
- Global disability scale for assessment of psychiatric disability evaluation assessment scale (IDEAS).[14] This schedule has been standardized on Indian patients and assesses disability on a number of domains.
- Unstructured questionnaire for perceived cause of domestic violence instruments have been developed for married women by the researcher.
- Descriptive and inferential statistics were used in order to analyze the data using SPSS version 16.

RESULTS

Sixty-five married women, 35 women with a psychotic illness and 30 non-psychotic illness were recruited into the study from the Sir Sunder Lal Hospital, Banaras Hindu University, Varanasi, Uttar Pradesh, India.

The demographic characteristics of sample are shown in Table 1A. The mean age of subjects of women with psychotic illness was 30.68 ± 5.74 years, and of non-psychotic illness was 31.60 ± 6.12 years. The mean years of education of psychotic illness was 10.31 ± 4.61 years, and

of non-psychotic illness was 8.73 ± 5.36 years. The mean duration of marriage of women with psychotic illness was 10.97 ± 7.28 years, and of non-psychotic illness was 14.00 ± 7.43 years. There was no significant difference between women with psychotic illness and two with respect to age, years of education, and duration of marriage (Table 1A).

All participant psychotic and non-psychotic illness women were Hindu. Marriages of all the patients were arranged. Majority of women with psychotic and non-psychotic illness came from rural background. A total of 72.3% of the subjects hailed from joint families, 27.7% were from nuclear families. Only 7.7% were employed in different occupations and 92.3% were housewife. Majority of husband 38.5% were clerical/shop-owner/farmer. 58.5% of the subjects belonged to upper middle socioeconomic status (SES), 38.8% belonged to lower middle SES. There was no significant difference between women with psychotic illness and two with respect to domicile, occupation (Home-makers *vs* non-home makers), SES and type of family of the subjects (Table 1B).

The mean age of onset of disease psychotic illness group was 25.49 ± 7.46 years and non-psychotic illness group was 28.83 ± 6.42 years. The mean duration of illness of psychotic illness was 46.86 ± 47.42 months and non-psychotic illness was 33.77 ± 36.60 months (Table 2A).

The most common diagnostic categories in psychotic illness was schizophrenia 48.5% These were followed by major depressive disorder with psychotic features 20% bipolar I disorder, most recent episode manic 14.2%; bipolar I disorder, single mania episode 8.5% and Brief

Table 1A: Demographic characteristics of the sample showing the mean and standard deviation

	Psychotic (n = 35)		*Non-psychotic (n = 30)*				
Variable	*Mean*	*SD*	*Mean*	*SD*	*t*	*df*	*p*
Age (years)	30.68	5.74	31.60	6.12	–0.62	63	0.537
Education (years)	10.31	4.61	8.73	5.36	1.27	63	0.206
Duration of marriage (years)	10.97	7.28	14.00	7.43	–1.65	63	0.103

Table 1B: Demographic characteristics of the sample showing frequency and percentage

	Psychotic (n = 35)		*Non-psychotic (n = 30)*		*Total (n = 65)*				
Variable	*n*	*%*	*n*	*%*	*n*	*%*	χ^2	*df*	*p*
Religion									
Hindu	35	100	30	100	65	100			
Type of marriage									
Arranged	35	100	30	100	65	100			
Domicile									
Rural	19	54.3	18	60.0	37	56.9	0.21	1	0.643
Urban	16	45.7	12	40.0	28	43.1			
Type of family									
Nuclear	10	28.6	22	73.3	18	27.7	0.02	1	0.864
Joint	25	71.4	8	26.7	47	72.3			
Occupation of wife									
Professional and semiprofessional/ skilled and semiskilled	2	5.7	3	10.0	5	7.7	0.41	1	0.518
Homemaker	33	94.3	27	90.0	60	92.3			
Occupation of husband									
Professional/Semiprofessional	1	2.9	4	13.3	5	7.7	5.68	5	0.338
Clerical/shop-owner/farmer	17	48.6	8	26.7	25	38.5			
Skilled worker	5	14.3	5	16.7	10	15.4			
Semi-skilled worker	7	20.0	7	23.3	14	21.5			
Unskilled worker	5	14.3	5	16.7	10	15.4			
Unemployed	0	0.0	1	3.3	1	1.5			
Socioeconomic status									
Upper	1	2.9	2	6.7	3	4.6	2.18	3	0.535
Upper middle	21	60.0	17	56.7	38	58.5			
Lower middle	12	34.3	8	26.7	20	38.8			
Upper lower	1	2.9	3	10.0	4	6.2			

Table 2A: Clinical characteristics of the sample: age of onset and duration of illness

	Psychotic (n = 35)		*Non-psychotic (n = 30)*				
Variable	*Mean*	*SD*	*Mean*	*SD*	*t*	*df*	*p*
Age of onset (years)	25.49	7.46	28.83	6.42	–1.92	63	0.059
Duration of illness (month)	46.86	47.42	33.77	36.60	0.85	63	0.396
Duration of treatment (month)	33.34	44.56	22.30	22.16	1.23	63	0.223

Table 2B: Clinical characteristics of sample

	Psychotic illness group (n = 35)			*Non-psychotic illness group (n = 30)*	
Variables	*n*	*%*	*Variables*	*n*	*%*
Diagnosis Schizophrenia	17	48.5	*Diagnosis* Generalized anxiety disorder	10	33.3
Major depressive disorder with psychotic features	7	20	Major depressive disorder without psychotic features	9	30
Bipolar I disorder, most recent episode manic	5	14.2	Conversion disorder	6	20
Bipolar I disorder, single mania episode	3	8.5	Obsessive compulsive disorder	3	10
Brief psychotic disorder	3	8.5	Dissociative disorder	2	6.6

Table 3: Domestic violence in married women with psychotic and non-psychotic illness

Domestic violence (spousal) in women: severity and pattern							
	Psychotic (n = 35)		*Non-psychotic (n = 30)*				
Variable	*Mean*	*SD*	*Mean*	*SD*	*t*	*df*	*p*
Domestic violence	23.28	22.96	09.03	17.30	2.78	63	0.007
Psychological	15.68	16.47	6.36	12.09	2.56	63	0.013
Physical	7.60	8.33	2.60	5.37	2.82	63	0.006

psychotic disorder 8.5%. Non-psychotic illness was generalized anxiety disorder 33.3% major depressive disorder without psychotic features 30%, conversion disorder 20%, obsessive compulsive disorder 10% and Dissociative disorder 6.6% (Table 2B).

Domestic violence was reported significantly more by women with psychotic illness (23.28 ± 22.96) than by non-psychotic illness (09.03 ± 17.30). The majority of participants was reported psychological violence was reported more in women with psychotic illness (15.68 ± 16.47) than in non-psychotic illness (6.36 ± 12.09) and physical violence was also reported significantly more in women with psychotic illness (7.60 ± 8.33) than in non-psychotic illness (2.60 ± 5.37). There was a statistically significant difference in severity of spousal violence reported between the two groups, more in psychotic illness than in non-psychotic illness, both for total/psychological and for physical violence (Table 3).

The mean disability of women with psychotic and non-psychotic illness was 2.48 ± 0.50 and 2.00 ± 0.58. The mean disability scores of psychotic illness group were significantly higher than corresponding scores of non-psychotic illness group (Table 4).

Table 4: Comparison of disability score in married women with psychotic and non-psychotic illness

Comparison of patient group on disability score						
	Psychotic (n = 35)		*Non-psychotic (n = 30)*			
Variable	*Mean*	*SD*	*Mean*	*SD*	*z*	*p*
Overall disability	2.48	0.50	2.00	0.58	–3.20	0.001

The distribution of patients with respect to causes, many women gave more than one cause. Not able to carry out domestic chores was the most common and reported by 83.7%. In about 46.5%, the cause was not being to be a good sex partner. This was followed by dowry, other family members complain about her behavior and by family and slow, unsatisfactory 34.8% each (Table 5).

DISCUSSION

The present study is from Sir Sunder Lal Hospital, Banaras Hindu University, Varanasi, Uttar Pradesh, India which caters to a huge population from Eastern Uttar Pradesh, Bihar, Madhya Pradesh and even Nepal. This region is densely populated with a relatively low level of literacy and psychological sophistication.

Table 5: Causes of domestic violence against women with psychotic and non-psychotic illness

Causes of domestic violence									
Variable	Psychotic illness (n = 28)		Non-psychotic illness (n = 15)		Total (n = 43)				
Presenting cause	n	%	n	%	n	%	χ^2	df	p
Domestic chores	23	82.1	13	86.6	36	83.7	3.27	1	0.070
Unable to be a good sex partner	12	42.8	8	53.3	20	46.5	0.44	1	0.507
Dowry	10	35.7	5	33.3	15	34.8	1.29	1	0.256
Other family members complain about her behavior	12	42.8	3	20.0	15	34.8	5.36	1	0.021
Slow, unsatisfactory	10	35.7	5	33.3	15	34.8	1.29	1	0.256

Majority of the abused women were found to remain silent about their experience of violence. The frequency, pattern and magnitude of domestic violence by husbands during the past years in patients with psychiatric morbidity were examined in comparison with psychotic and non-psychotic women. Domestic violence was significantly higher in the group of women with psychotic illness than in non-psychotic illness. The findings suggest a high prevalence of experiences of domestic violence among psychiatric patients.[13]

Most of the psychiatric ill women were suffering from schizophrenia followed by depression. Avdibegovic found that women who experienced domestic abuse showed symptoms of different psychiatric problem.[1]

The most common cause of domestic violence was domestic chores followed by unable to be a good sex partner, dowry, other family members complain about her behavior and slow-unsatisfactory. This finding suggested according to Kaur, the most common cause of domestic violence was dissatisfaction of dowry, arguing with the partner, refusing to have sex with him and not cooking properly or on time.[8]

The findings of this study have practical implications. First, there is little recognition among health planners that psychiatric morbidity could be a cause of domestic violence. Second, domestic violence can be prevented by early detection and treatment. The protection of women from Domestic Violence Act 2005 does not recognize psychiatric morbidity in women as a cause of domestic violence.[15] In the Act, there is provision for a special order 'Not to consume alcohol or drugs which lead to domestic violence in the past', but none for medical treatment of the same.

The strength of the study is the use standardized culturally appropriate instruments for evaluation.

Global disability and cause of domestic violence of women with psychotic illness is more than women with non-psychotic illness.

CONCLUSION

On the basis of this study, we can say that domestic violence was more in women with psychotic illness compare to non-psychotic illness. The predominant form of violence was found to be psychological violence. There is no single factor to account for violence perpetrated against women. Domestic chore was the main factor to cause of domestic violence. We would recommend developing a better program to be developed for bringing about wider awareness in psychiatric ill women and also marital counseling of both partners to prevent domestic violence.

Education and public enlightenment campaign on evils of domestic violence should be encouraged and organized by guidance counselors, social welfare workers, religious leaders and the government. Government should establish marriage counseling units in all local government areas in the state where couple or couples would go for counseling against domestic violence.

The healthcare personnel should be given an opportunity to update their knowledge regarding domestic violence and need education for domestic violence and cessation, so that they can help the women to protect/prevent domestic violence.

LIMITATIONS OF THE STUDY

- This study was on hospital-based sample. It would have been more realistic to have the sample from the community.
- This study focus on women with psychotic and non-psychotic illness. There is a need to focus studies

on men, as the men are the active partner in act of domestic violence. Focused on causes of domestic violence in men with psychotic and non-psychotic illness are likely to be more effective.

IMPLICATIONS FOR THE STUDY

- The findings provide robust evidence for greater degree of domestic violence in women with mental illness than nonmental illness and causes of domestic violence in women with mental illness are more non mental illness women.
- The implication of this finding is that prevention of domestic violence must involve engagement with both sides of a relationship. Coordinated action seems to be needed at many levels to ensure that material efforts to improve the status of women are coupled with a focus on men to promote acceptance of the need for change, whether at an individual level, or through interventions focusing on men with low socioeconomic status.
- There is an enormous potential for detailed assessments of intervention strategies, not only to guide future policy, but also to provide insights into interrelations between causal factors and develop knowledge of the causes of domestic violence.
- Further research is needed, however, to investigate which interventions are effective in reducing domestic violence experienced by women with mental disorders and how to improve mental health after the abuse has stopped.

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ORIGINAL ARTICLE

Effect of Selective Serotonin Reuptake Inhibitors on Psychomotor Function in Patients of Depression: A Comparative Study of Sertraline and Fluoxetine

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ABSTRACT

Aims and objectives: Depression is a most common and widespread of all psychiatric disorders. Treatment of depression includes the use of antidepressants, commonly used clinically, such as tricyclic antidepressants, selective serotonin reuptake inhibitors, selective norepinephrine reuptake inhibitor, and monoamine oxidase inhibitors. Certain antidepressants apart from improvement in the symptoms found to have detrimental effect on cognitive and psychomotor functions. Objective of this study was to assess and to compare the effect of sertraline and fluoxetine on cognitive and psychomotor functions.

Materials and methods: Effect of sertraline and fluoxetine on psychomotor function was assessed by using critical flicker fusion frequency (CFF) and reaction time (RT) in patients of mild to moderate depression at the end of 2nd and 4th week of monotherapy.

Results: Patients in both the group have their RT remained significantly higher ($p < 0.001$) in comparison with control and CFF remained significantly lower at the end of both the week except sertraline group in which CFF did not differ significantly from control at the end of 4th week. There was a significant rise in CFF ($p < 0.05$) in sertraline group as compared to fluoxetine. Sertraline showed a significant improvement ($p < 0.01$) in visual reaction time (VRT) at both the follow-ups and auditory reaction time (ART) ($p < 0.01$) at 4th week of monotherapy. Both the groups did not differ with respect to their effect on choice reaction time (CRT).

Conclusion: Findings of this study support the use of sertraline which had shown less impairment of psychomotor function in patients of depression as compared to fluoxetine, in special subgroups of population who operate machinery, drive vehicle or require alertness for the work.

Keywords: Antidepressants, Cognitive functions, Critical flicker fusion frequency, Reaction time.

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INTRODUCTION

Depression is a most common and widespread of all psychiatric disorders characterized by depressed mood for at least 2 weeks and or loss of interest or pleasure in most of the routine activities. In addition, depression is characterized by disturbances in sleep, appetite as well as deficit in cognition; thoughts of guilt, worthlessness and suicide are also common.[1] Antidepressants commonly used clinically are tricyclic antidepressants, selective serotonin reuptake inhibitors, selective norepinephrine reuptake inhibitor, and monoamine oxidase inhibitors.

Depression related cognitive impairment is a condition that is under-recognized, undiagnosed and undertreated.[2] Cognitive impairment ranges from deficit in short-term, long-term memory or alteration in decision making process and impairment of information processing.

The largest population-based study to date of late onset depressive illness (65–84 years) documented severe cognitive impairment in 10% of depressed patients.[3] Approximately 70% of elderly depressed patients have measurable cognitive deficits, although a physician may be unaware of any overt signs.[4] It is well established that antidepressants can improve patient's well-being and functioning but many have demonstrable detrimental effect on range of cognitive functions. The optimum profile of antidepressant includes no detrimental effect on cognitive and psychomotor functions.[5]

With moves toward continuation and maintenance therapy for depression antidepressants with relatively non-sedating, non-cognition impairing profiles, such as selective serotonin reuptake inhibitors (SSRIs) are preferred in patients of depression.[6] However, antidepressant like fluoxetine and other SSRI's are known to improve cognition and memory in some studies,[7,8] whereas in contrast, in some studies cognition and memory parameters have shown decline.[9]

There are differences emerging amongst the SSRI group with respect to their effects on cognitive and psychomotor function. In another study, fluoxetine has demonstrated significant impairment of sustained attention in healthy volunteers.[10] Sertraline has demonstrated cognition enhancing effects in healthy volunteers.[11]

The lack of a placebocontrol in many of the studies, particularly those in depressed patients, means that the apparent differences amongst SSRIs in their cognitive and psychomotor effects require further confirmation. Use of these antidepressants having cognitive and psychomotor function impairing properties may raise concern amongst employees of some critical job that require high level of alertness such as drivers,[12] students, factory workers, machinery operators.[13] Since, antidepressants have to be used on chronic basis, it is important to evaluate the effect of these antidepressants on cognitive and psychomotor functions, such assessment would help in selecting a particular drug based on the individual patient requirements.

This study has been undertaken to determine the effect of commonly used antidepressants such as SSRIs on cognitive and psychomotor functions and to compare the effect of sertraline *vs* fluoxetine with healthy unmedicated adults as control group.

MATERIALS AND METHODS

The present study was an open label prospective comparative clinical study. It was carried out from Jan 2013-June 2013 and was approved by Institutional Ethics committee. Aim of the study was to evaluate the effect of commonly prescribed SSRIs sertraline and fluoxetine on cognitive and psychomotor functions. Assessment was carried out using CFF and RT. The present study included objective measures from various divisions of psychomotor performance, viz. sensory, central integration, and motor component.

Inclusion and Exclusion Criteria

Inclusion Criteria

(i) Patients of either sex; (ii) Patients within the age limit 18 to 60 years; (iii) A known cases of mild to moderate depression diagnosed by DSM-IV criteria and HDRS scale;[14] (iv) Patients who were on monotherapy with either SSRIs (fluoxetine 20 mg, sertraline 50 mg); and (v) Patients who were willing to participate in the study and willing to give written informed.

Exclusion Criteria

(a) Patients who were on any other medications (antihypertensive, sedative and systemic steroid, etc.) that are known to affect cognitive and psychomotor functions; (b) Patients with serious systemic disorders (diabetes, hypertension, etc.); (c) Patients with any psychiatric illness or any other CNS disorder that will interfere with cognitive and psychomotor functions except depression; (d) Patients who were not willing to participate and not given written informed consent; and (e) Patients with severe depression with HDRS score >17.

Outpatient department patients diagnosed with depression and meeting with inclusion criteria were enrolled in the study. Age and sex matched healthy adult volunteers were assigned to control group. Aim, procedure of the study and tests were explained to the study participants. The enrolled patients were explained about the importance of this study and written informed consents were obtained.

Each patient was familiarized with CFF and RT tests. Tests were carried out between 10:00 am and 1:00 pm. Before beginning of the study, patient's vital data, such as name, age, sex, educational status, occupation were noted on first visit. Other things like symptoms, illness duration, past history family history, past drug history were also noted. Vital data and details of systemic examination were recorded. A note of the diagnosis and treatment was recorded in the performa during each visit. General and systemic examinations were carried out to exclude any systemic disease. Enrolled subjects were grouped in 3 as follows:

Test Performed

Critical flicker fusion frequency test; and (ii) Reaction time performance test: (a) Visual reaction time; (b) Auditory reaction time; and (c) Choice reaction time.

Critical Flicker-Fusion Test

It was assessed by the critical flicker-fusion apparatus (Techno Electronics, LalbaughLucknow-226001). The apparatus is housed in a metal cabinet having two sloping sides the light source flickers at the rate set by the experimenter. The flicker frequency range of the instrument is 5 to 50 Hz the CFF (Fig. 1).

Subjects were asked to indicate when a red-light-emitting flickering source increasing in frequency, is perceived to become a continuous signal. They were also required to distinguish the threshold at which a flickering signal was perceived from a continuous signal, when frequency decreased. This fusion and flicker are a reliable measure of cortical alertness and arousal and reasonably stable in a given subject. Decreases in thresholds is indicative of altered CNS function.[15]

Determination of 'Critical Fusion Frequency

The 'flicker per second' knob of the instrument was kept at minimum frequency of 5 Hz. The volunteers

Fig. 1: Techno flicker-fusion apparatus

were told to view a flickering light source through the eyepiece. They were allowed adaptation to the least flicker frequency for 1 minute. Then frequency was increased slowly by rotating the flicks per second knob clockwise. The frequency increase was stopped as soon as patient responded by pressing the response switch, when he saw fusion, i.e. no more flickering or a steady light source. Frequency from the dial setting was noted. Three such readings were taken and the score was calculated as the mean of these 3 readings.[16]

Determination of 'Critical Flicker Frequency'

After determination of critical fusion frequency, the flicks per second knob was adjusted to maximum frequency of 50 Hz, after 1 minute adaptation frequency was decreased slowly, by rotating the flicks per second knob anticlockwise. The frequency reduction was stopped as soon as the subject responded when he saw flickering. Three reading were taken and the score was calculated as the mean of these 3 readings.[16]

Mean of both (a) and (b) was then calculated.

Mean CFF value is decreases with age, also with the antidepressants impairing cognitive and psychomotor function and hypnotics,[17] while CNS stimulant drugs increases critical flicker fusion frequency.[17,18]

Reaction Time Performance Test

Reaction time performance test was assessed using (Digital Display Multiple Choice 4-visual+4- Aural Type MCR-444) Lalbaugh Lucknow-2220. It measures reaction time, i.e. time interval after which subject responds to stimulus either visual/auditory.

Four different stimuli of different colors and four aural stimuli of different tones, with independent operation were provided. Chronoscope is a four segment LED Display with a minimum count of 00.10 seconds and maximum of 99.99 seconds.

The techno digital display multiple choice is a compact portable unit with sloping operating panel. On both sides for ease of operation, a removable partition effectively shields the operation side from each side. It operates from 220V 50 Hz AC (Fig. 2).

Experimenter's side contains (a) red, green, yellow and amber colored LED's lights or any four different colors, (b) The bottom row has eight press buttons four for visual stimuli four for auditory stimuli. Subject's side contains: red, green, yellow and amber colored visual stimuli (or matching different colors) and eight press buttons four for visual four for aural stimuli.

Sensory component is an important aspect of psychomotor performance. Detection, perception, and recognition of a stimulus are three levels of information processing which together account for the majority of sensory activity. Thus, reaction time performance measure the processing of sensory information.

Reaction time is impaired/increased with drugs declining cognitive functions, depression, with increasing age[18] (time taken to respond to stimuli is increased with cognitive and psychomotor impairment could be due to drug or depression itself). Certain antidepressants, caffeine[19] and CNS stimulant, such as amphetamine produces reduction reaction time.[20]

In the present study, patients were included in such a way so as to exclude any extraneous influence on psychomotor function like drugs (e.g. antiepileptic, sedative-hypnotics, antipsychotic) and diseases (serious systemic disorder, for example diabetes mellitus.) Thus, the changes observed in the study if any could be attributed to two factors, first the effect of drugs on psychomotor function parameters and second improvement in the disease, i.e. depression.

STATISTICAL ANALYSIS

Data presented using charts and descriptive statistics, such as mean, standard deviation (SD), standard error (SE).

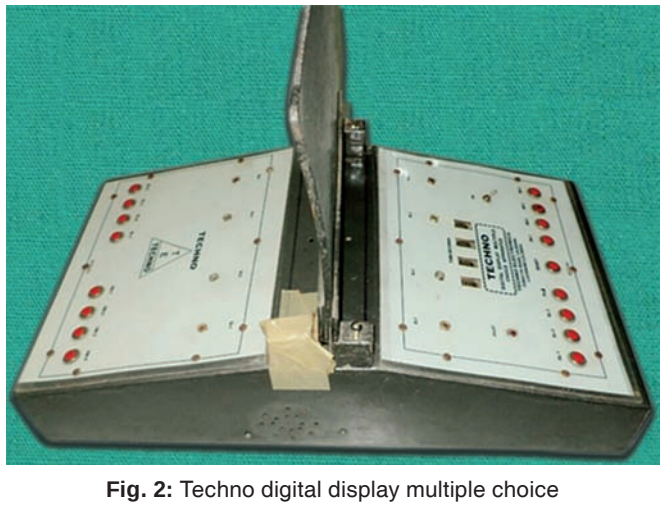

Fig. 2: Techno digital display multiple choice

Further statistical analysis was being done using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. The significance level was set at 5%, p-value less than 0.05 was considered as a significant.

RESULTS

In this study, there were 55% males and 45% females. Out of 60 study subjects 55% were in the age group of (18-40) and 45% were in the age group of (41–60) (Table 1).

At 2nd and 4th week of monotherapy when both the drug groups compared with control, mean CFF, mean VRT and mean CRT was decreased significantly ($p < 0.001$), except sertraline at 4th week in which CFF did not differ significantly from control group (Table 2).

Mean CFF in sertraline group increased significantly when compared with fluoxetine ($p < 0.05$) at 2nd week (31.68 ± 0.21) and 4th week (33.1 ± 0.20) of treatment. Effect of sertraline on visual reaction time did not differ significantly from fluoxetine. However, it was reduced (159.77 ± 4.44) significantly at 4th week ($p < 0.05$). Mean ART was significantly low in sertraline group when compared with fluoxetine at the end of both 2nd (209.85 ± 2.24) and 4th week (146.33 ± 4.51) of treatment. Effect of sertraline on choice reaction time did not differ significantly from fluoxetineat both the follow-ups.

Table 1: Sociodemographic profile of study participants

	Sertraline (n = 20)	*Fluoxetine (n = 20)*	*Control (n = 20)*
Sex			
Male	12 (60)	10 (50)	11 (55)
Female	8 (40)	10 (50)	9 (45)
Age group			
18–40	12 (60)	10 (50)	11 (55)
41–60	8 (40)	10 (50)	9 (45)
Occupation			
Student	3 (15)	5 (25)	4 (20)
Household worker	4 (20)	3 (15)	3 (15)
Factory worker	5 (25)	4 (20)	3 (15)
Laborer	4 (20)	4 (20)	4 (20)
Office worker	3 (15)	2 (10)	4 (20)
Others	1 (5)	2 (10)	2 (10)

Note: Figures in parenthesis show percentages

DISCUSSION

Depression affects 121 million people worldwide; it has life time prevalence of 16.2% and 12 months prevalence of 6.6% in developed countries,[21] and is a leading cause of disability worldwide. As far as the burden of depression in India is concerned, many studies have estimated the prevalence of depression in community samples and the prevalence rates have varied from 1.7 to 74 per thousand in Indian population.[22,23] The largest population-based study to date of late onset depressive illness (65–84 years) documented severe cognitive impairment in 10% of depressed patients.[3] Approximately, 70% of elderly depressed patients have measurable cognitive deficits, although a physician may be unaware of any overt signs.[4] It is well established that antidepressants can improve patient's well-being and functioning but many have demonstrable detrimental effect on range of cognitive functions. The optimum profile of antidepressant includes no detrimental effect on cognitive and psychomotor functions.[5]

Studies are available which show the effect of antidepressants on cognitive and psychomotor function but most of these studies are single-dose studies and healthy volunteers were used as a study subjects.[24] Few studies reveal the effect of selective serotonin reuptake inhibitors on cognitive and psychomotor performance in depressed patients.[24,26]

In the present study, at 2nd and 4th week of monotherapy, when both the drug groups compared with control, mean CFF of all patients was significantly less except sertraline group in which CFF did not differ significantly from control at 4th week. Present results considering the effect of fluoxetine on cognitive and psychomotor performance in comparison with control as measured by CFF is in commensurate with findings of Sabbe et al (1997),[25] in which they had assessed sensory-motor programming, coordination, initiation and execution of muscle commands and feedback processing. The performances of patients receiving fluoxetine were compared to a control group of

Table 2: Effect on CFF and RT at the end of 2nd and 4th week between various groups (mean ± SEM)

Test ↓ *Group* →		*Sertraline (n = 20)*		*Fluoxetine (n = 20)*	
Time interval	*Control (n = 20)*	*2nd week*	*4th week*	*2nd week*	*4th week*
CFF	33.34 ± 0.17	31.68 ± 0.21[ac]	33.1 ± 0.20[ac]	28.43 ± 0.25[b]	28.92 ± 0.22[b]
VRT	163.06 ± 2.69	222.35 ± 2.26[a]	209.85 ± 2.24[ad]	232.66 ± 3.83[b]	230.30 ± 3.97[b]
ART	111.27 ± 4.48	159.77 ± 4.44[ae]	146.33 ± 4.51[ae]	212.25 ± 3.11[b]	207.20 ± 2.97[b]
CRT	335.20 ± 12.95	388.36 ± 3.71[a]	375.20 ± 4.11	411.40 ± 3.24[b]	408.50 ± 3.33[b]

Time in millisecond (msc) and frequency in Hz

CFF: Critical flicker fusion frequency; RT: Reaction time; VRT: Visual reaction time; ART: Auditory reaction time; CRT: Choice reaction time; [a]$p < 0.001$ as compared with control; [b]$p < 0.001$ as compared with control at both the weeks, [c]$p < 0.05$ as compared with fluoxetine at 4th week, [d]$p < 0.01$ as compared with fluoxetine at both the weeks, [e]$p < 0.01$ as compared with fluoxetine at the end of 2nd and 4th week

22 individuals. The significant slowing of motor processes in the depressed in-patients decreased but did not disappear after treatment. At the end of treatment significant differences persisted between the patient group and the control group. Significant slowing of motor processes in depressed inpatients receiving fluoxetine decreased but did not disappear at the end of 6th week.[25]

Improvement in mean CFF (33.1 ± 0.20) in sertraline group at 4th week is in agreement with the study done by Schrijvers (2009), in which they reported that the patients psychomotor slowing had improved after 6 weeks on sertraline reflected by reductions in initiation and movement times on simple line and figure copying task and decreased initiation times for complex figure copying task relative to their baseline outcome.[26]

Mean (VRT, ART and CRT) of all drug groups were remained significantly on higher side as compared to control. Mean CFF in sertraline group was increased as compared to fluoxetine at the end of 2nd (31.68 ± 0.21) and 4th week (33.1 ± 0.20). It is in consistence with findings of the study done by Newhouse (1996).[27] In their study, there was a significant improvement in the parameters of cognition such as DSST in sertraline group relative to baseline from week 1 and relative to fluoxetine at 6th and 12th week. Fluoxetine only significantly improved DSST relative to baseline at 12th week. Both the treatment improved shopping list task however the improvement was greater in sertraline group and it was significantly greater than fluoxetine 6th week.

Effect of sertraline on visual reaction time did not differ significantly from fluoxetine group at 2nd week. However; it was significantly low at 4th week of monotherapy. Mean ART in sertraline group was significantly low when compared to fluoxetine at both the follow-ups. Effect of both the groups on choice reaction time didnot differ significantly from each other at 2nd and 4th week.

Sertraline showed a significant improvement in cognitive and psychomotor functions as far as its effect on parameters, such as CFF and RT were concerned.

CONCLUSION

Findings of this study support the use of sertraline which had shown less impairment of psychomotor function in patients of mild to moderate depression as compared to fluoxetine. Drugs which have low behavioral toxicity should therefore be preferred as they are less disruptive of patients everyday activities produce better quality of life. It may be preferred in patients who operate machinery, drive vehicle or require alertness for the work. However, our findings need confirmation by using larger number of patients with repeated follow-up.

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ORIGINAL ARTICLE

A Comparative Evaluation of Levobupivacaine Hydrochloride and Levobupivacaine Hydrochloride with Dexmedetomidine in Epidural Anesthesia and Postoperative Pain Relief undergoing Infraumbilical Surgeries

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ABSTRACT

Epidural anesthesia is a versatile technique which is widely used for surgeries for providing intra- and postoperative analgesia. Several adjuvants have been used to prolong the action of the local anesthetic agent used.

Aims and objectives: We performed prospective randomized, double blinded controlled study on 90 patients to compare the effects of adding of dexmedetomidine to levobupivacaine in prolonging the analgesia produced by epidural levobupivacaine alone in patients undergoing infraumbilical surgeries and also compared the duration of motor block and sedation scores.

Materials and methods: Ninety American Society of Anesthesiologists (ASA) I and II patients (18–60 years), undergoing infraumbilical surgery, were prospectively randomized to one of two groups to receive epidural anesthesia with 17 ml of 0.5% levobupivacaine + 3 ml of normal saline (group L) or epidural anesthesia with 17 ml of 0.5% levobupivacaine with 75 μg (0.75 ml) of dexmedetomidine + 2.25 ml of normal saline (group LD). Various parameters hemodynamic changes, onset time of sensory and motor blockade, highest level of sensory blockade, duration of sensory and motor block, postoperative pain using visual analog scale (VAS) score, and any side-effects were recorded and data were statistical analyzed using student's t-test by statistics calculator SPSS software.

Results: The two study groups were similar for mean age, weight and duration of surgery. Mean duration of analgesia was significantly longer in group LD (438.33 ± 38.72 min) than in group L (271.2 ± 23.77 min); $p < 0.05$. Onset time of sensory and motor blockade was significantly less in group LD as compared to group L; $p < 0.05$. Duration of sensory and motor block was significantly higher in group LD when compared to group L ($p < 0.05$). More sedation was observed in group LD.

Conclusion: Dexmedetomidine in a dose of 75 μg added as an adjuvant to 0.5% levobupivacaine for epidural anesthesia, during infraumbilical surgeries, prolongs the duration of analgesia of levobupivacaine and increases postoperative sedation, without any other adverse effects.

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INTRODUCTION

Epidural anesthesia is one of the most familiar and widely used technique for providing not only perioperative surgical anesthesia but also postoperative analgesia in lower abdominal and limb surgeries.[1] Early postoperative ambulation and rehabilitation with minimally associated pain and discomfort are the most desirable features in modern infraumbilical and lower limb surgeries.[2-4] The intense sensory and motor blockade, along with continuous supine position for a prolonged duration and the inability to move the body during regional anesthesia brings a feeling of discomfort and phobia in many of the patients.[5] The high cephalic spread of local anesthetics may be significant but still its quality sometimes may not correlate with the level of sensory analgesia.[6] At this stage, the impulsive use of large doses of sedation or even general anesthesia with mask defeats the novel purpose of regional anesthesia whereby a continuous verbal contact with the patient is lost. Sedation, stable hemodynamics and an ability to provide smooth and prolonged postoperative analgesia are the main desirable qualities of an adjuvant in neuraxial anesthesia.

Various adjuvants, like epinephrine, fentanyl, dexamethasone, clonidine[7-10] when added to levobupivacaine were found to prolong the duration of analgesia dexmedetomidine is a new addition to the class of alpha-2 agonist with varied beneficial effects when administered via epidural route. It acts on both pre- and post-synaptic sympathetic nerve terminal and central nervous system, thereby decreasing the sympathetic outflow and norepinephrine release causing sedative, antianxiety, analgesic, sympatholytic and hemodynamic effects.

Dexmedetomidine does cause a manageable hypotension and bradycardia which is treatable but the unraveling feature of this drug is the lack of opioid related side-effects like respiratory depression, pruritus, nausea and vomiting.

AIMS AND OBJECTIVES

To study and compare the effects and efficacy of levobupivacaine and levobupivacaine with dexmedetomidine in epidural anesthesia with reference to the onset and duration of sensory loss, the onset and duration of motor loss, the duration of analgesia and to observe any untoward complications.

MATERIALS AND METHODS

After approval from the Ethics Committee, a prospective randomized, double blinded controlled study was performed on total of 90 patients of both genders aged 18 to 60 years, with physical status American Society of Anesthesiologist (ASA) I and II, who underwent infraumbilical surgeries, i.e. total knee replacements, total hip replacements, appendicectomy, fistulectomy, herniorrhaphy, etc. lasting not more than 120 minutes. Patients with peripheral or central neurological disease, raised intracranial tension, valvular heart diseases, significant ECG changes, renal disease, endocrinal disease, metabolic diseases, hepatic disease, coagulopathy and bleeding diathesis, body weight of >100 and <45 kg and height of <145 cm were excluded from this study. Patients were divided randomly into two groups: group LD (n = 45): Levobupivacaine with dexmedetomidine and group L (n = 45): Levobupivacaine alone. All patients were premedicated with injection glycopyrrolate 0.2 mg IM. Thirty minutes before surgery, patients were thoroughly counseled during the preoperative evaluation and well informed consent obtained.

In the operation room, a venous access was secured with 18 G cannula, and all the patients were prehydrated with 10 ml/kg of lactated Ringer's solution. Heart rate (HR), electrocardiography (ECG), Noninvasive blood pressure (NIBP), and pulse oximetry (SPO_2) were monitored and recorded.

Epidural anesthesia was administered via 16 G Touhy needle, with patients in the sitting position at L3 to L4 interspace and epidural space was identified by loss of resistance technique. A test dose of 3 ml of 2% lignocaine with adrenaline was administered into the epidural space. The study solutions were prepared by anesthesia technician who was given written instructions and was unaware of study design to avoid biasing. The following solutions were randomly administered group L (n = 45) 17 ml of levobupivacaine hydrochloride 0.5% with 3 ml normal saline injected epidurally. Group LD (n = 45) 17 ml of levobupivacaine hydrochloride 0.5 with 75 μg (0.75 ml) of dexmedetomidine and 2.25 ml of normal saline injected epidurally. The pin prick method was used to asses onset time of sensory blockade, whilst Modified Bromage scale (0 = no block, 1 = inability to raise extended leg, 2 = inability to flex knee, and 3 = inability to flex ankle and foot) was used to measure onset time of motor blockade, highest level of sensory and motor blockade were observed immediately after administration of epidural block. Duration of sensory blockade, i.e. time taken for sensory block to regress by two segments below the highest level was noted. Duration of analgesia, i.e. time taken from onset of sensory block to the first request of rescue analgesia was observed. Analgesia was assessed by VAS score at the time of first analgesia request only. Side effects and complications, such as nausea, vomiting, hypotension, bradycardia and shivering due to drugs were notified as well.

Sedation was assessed using 6 points Ramsay Sedation score (1 = Patient anxious and agitated or restless, 2 = patient co-operative, oriented and tranquil, 3 = patient responds to commands only, 4 = patient exhibits brisk response to light glabellar tap or loud auditory stimulus, 5 = patient exhibits a sluggish response to light glabellar tap or loud auditory stimulus, and 6 = patient exhibits no response).

STATISTICAL METHODS

The data were analyzed using student's t-test by statistics calculator SPSS software. Student's t-test for inter group comparison. All the variables are expressed as mean $\pm$ standard deviation. The value of $p > 0.05$ was taken to be statistically insignificant and $p < 0.05$ taken as statistically significant. The sample size of 45 in each group was calculated using the prevalence of postoperative pain percentage in infraumbilical surgeries with power of 80% and alpha error of 0.05.

All the data are expressed as mean and standard deviation unless specified.

RESULTS

The demographic characteristics and duration of surgery in both the groups exhibited marked similarities and did not show any statistical significant variance ($p > 0.05$) (Table 1).

Onset time of sensory blockade in groups L and LD were 15.96 ± 2.41 and 6.78 ± 1.38 minutes respectively. The mean (±SD) onset time of motor blockade was 19.89 ± 2.26 minutes and 9.73 ± 1.40 minutes in groups L and LD respectively, which was significantly lower in group LD as compared to group L (Table 2).

Table 1: Demographic profile and duration of surgery

Variables	*Group L (mean ± SD)*	*Group LD (mean ± SD)*	*p-value*
Age (years)	36.4 ± 11.59	40.02 ± 13.91	0.183
Weight (kg)	64.51 ± 4.69	64.89 ± 5.05	0.714
Height (cm)	166.36 ± 5.03	167.29 ± 4.88	0.374
Duration of surgery (min)	79.84 ± 18.26	86.78 ± 19.72	0.087
Sex ratio (M:F)	36:9	34:11	1.000

*p < 0.05; statistically significant

Table 2: Perioperative characteristics in both the groups

Parameters	*Group L*	*Group LD*	*p-value*
Onset time of sensory blockade (min)	15.96 ± 2.41	6.78 ± 1.38	0.000*
Onset time of motor blockade (min)	19.89 ± 2.26	9.73 ± 1.40	0.000*
Highest dermatome level of sensory block (T4)	55.5%	75.6%	0.000*
Duration of sensory blockade (min)	173.84 ± 12.92	244.78 ± 15.11	0.000*
Duration of motor blockade (min)	199 ± 12.95	279 ± 16.12	0.000*
Duration of analgesia (min)	271.2 ± 23.77	438.33 ± 38.72	0.000*

*p < 0.05; statistically significant

The maximum sensory level achieved by both the groups is T4, level. The 55.5% of patients in group L and 75.6% of patients in group LD attained the highest sensory level of T4 whereas 37.8% of patients in group L and 24.4% of patients in group LD attained the level of T6. Only 6.7% of patients in group L and none in group LD attained the level of T8, which was significantly higher in group LD as compared to group L (Table 2).

Duration of sensory blockade was 173.84 ± 12.92 minutes, 244.78 ± 15.11 minutes in groups L and LD respectively and the (mean ± SD), duration of motor blockade was 199 ± 12.95 minutes in group L and 279 ± 16.12 minutes in group LD, which was significantly higher in group LD as compared to group L (Table 2).

Duration of analgesia in group L was 271.2 ± 23.77 minutes and in group LD was 438.33 ± 38.72 minutes, which was significantly higher in group LD as compared to group L (Table 2). 82.2% patients in group L and 48.9% patients in group LD had Ramsay Sedation Score of 1, whereas 17.8 and 51.1% patients in group L and LD had Ramsay Sedation Score of 2 respectively. Complications due to drug among both the groups are shown in (Table 3).

Table 3: Comparison of side effects in patients treated with levobupivacaine with (Group LD) or without dexmedetomidine (Group L)

	Group L		*Group LD*	
Complications	*No.*	*%*	*No.*	*%*
Nausea and vomiting	1	2.22	—	—
Hypotension	1	2.22	2	4.44
Bradycardia	—	—	4	8.88
Shivering	4	8.88	—	—
Dyspnea	—	—	—	—
Chest pain	—	—	—	—
Respiratory depression	—	—	—	—

DISCUSSION

Epidural analgesia is now not in the nascent stage. Every anesthesiologist is administering it world widely for post-operative pain relief as it offers better patient outcome.[4]

This epidural analgesia offers superior pain relief and early mobilization especially when local anesthetic dose is combined with an adjuvant as compared to local anesthetic used alone.[2] Opioids are most commonly used as adjuvants and are associated with side effects, such as respiratory depression, nausea, urinary retention and pruritis.[7] So various options including alpha-2 agonist are being extensively evaluated as an alternative to benzodiazepines, opioids and ketamine.[11-13] Alpha-2 agonist is clinically used widely as its epidural administration is associated with sedation, analgesia, anxiolysis, hypnosis and sympatholysis.[9,10]

The synergism between epidural local anesthetics and opioids is well established but evidence regarding combination of local anesthetics with dexmedetomidine through epidural route is scarce in literature.[14,15] Thus, study was conducted to compare the analgesic efficacy, perioperatively, as well as to observe the sedative effect of the dexmedetomidine (alpha-2 agonist) administered epidurally.

The demographic profile in the present study was comparable with respect to age, body weight, sex, height, duration of surgery; throughout the perioperative period patient were calm and comfortable with better sedation score of 2 in 51.1% of patients in group LD and 17.8% of patients in group L. Thus, showing dexmedetomidine produces better sedation when used epidurally.

The mean onset of sensory blockade was significantly (p = 0.000) shorter in group LD (6.78 ± 1.38) minutes as compared to group L (15.96 ± 2.41) minutes. The mean onset of motor blockade was also significantly (p = 0.000) shorter in group LD (9.73 ± 1.40) minutes as compared to group L (19.89 ± 2.26) minutes, further stating that dexmedetomidine promotes faster onset compared to levobupivacaine alone.

The mean duration of sensory blockade was significantly (p = 0.000) longer in group LD (244.78 ± 15.11) as compared to group L (173.84 ± 12.92) minutes. Similarly, the mean duration of motor blockade was significantly (p = 0.000) longer in group LD (279 ± 16.12) minutes as compared to group L (199 ± 12.95) minutes.

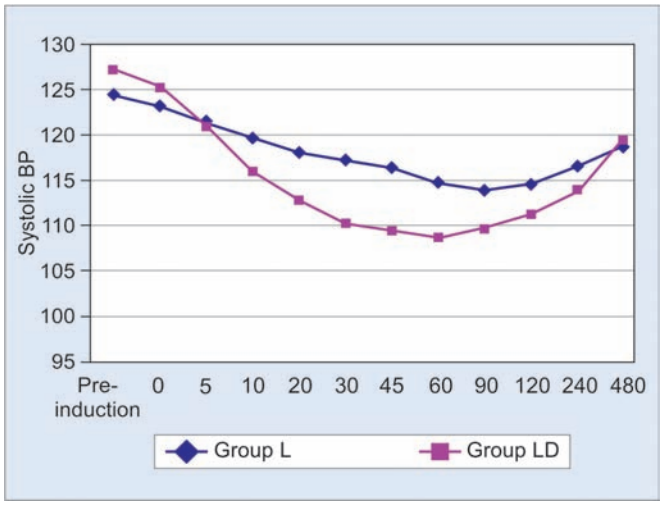
The mean duration of analgesia was significantly (p = 0.00) longer in group LD (438.33 ± 38.72) minutes as compared to group L (271.2 ± 23.77) minutes showing that dexmedetomidine gives a better perioperative analgesia profile when added to levobupivacaine as compared to levobupivacaine alone. The analgesic effect of dexmedetomidine is caused by the stimulation of dorsal root neuron of spinal cord, where alpha-2 agonists inhibit the release of norephinephrine.[16]

Throughout the surgery, patients were calm and compose among both the groups but sedation scores were significantly better in the LD group as compared to group L. The mean (± SD) VAS score at time of first rescue analgesia request was 47.91 ± 10.04 in group L while it was 40.04 ± 5.65 in group LD.

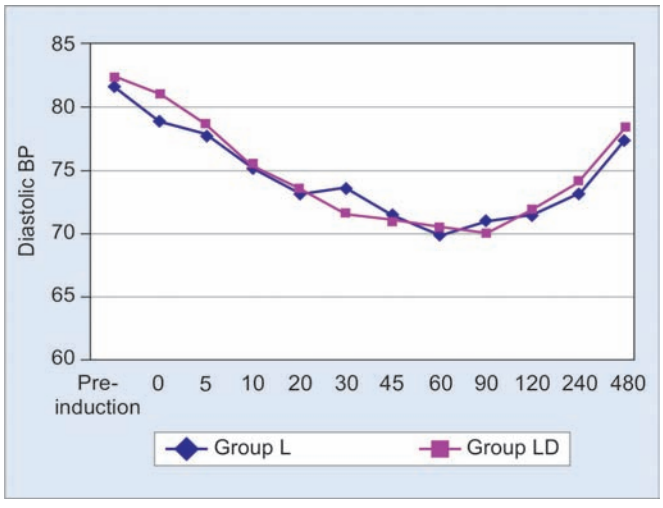
Dexmedetomidine in group LD provided a higher dermatomal level of (T4) in about 75.6% of patients as compared to 55.5% patients in group L.

The cardiorespiratory parameters mean HR (Graph 1), systolic BP (Graph 2), diastolic BP (Graph 3), remained stable throughout the study period which reaffirms the established effects of alpha-2 agonists in providing a hemodynamically stable perioperatively.

None of the patients had shivering in group LD as compare to 8.8% of patients in group L. Bradycardia was observed in 8.8% of patients of group LD and none in group L (Table 3). Bradycardia in patients having HR of < 50 bpm were treated with injection atropine 0.6 mg intravenously. Bradycardia in group LD can be attributed to dexmedetomidine, an alpha-2 agonist which can again be explained on the basis of its central action, whereby it decreases the sympathetic outflow and norepinephrine release.[16-18]



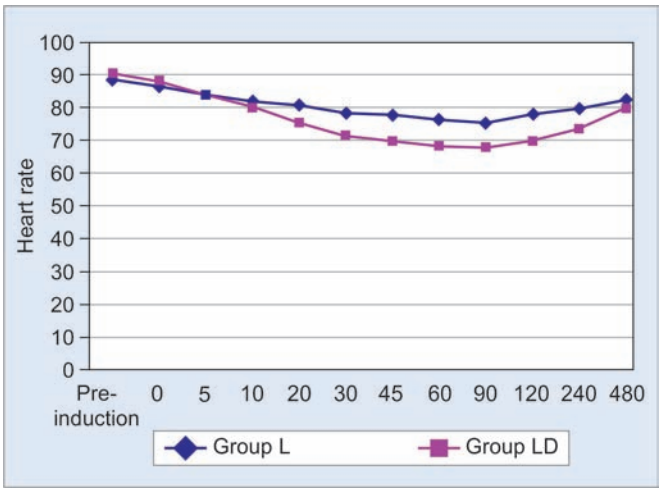
Graph 2: Comparison of mean systolic blood pressure (mm Hg) between two groups at different time intervals



Graph 3: Comparison of mean diastolic blood pressure (mm Hg) at different time intervals in both the groups

CONCLUSION

Epidural administration of dexmedetomidine (75 μg) with levobupivacaine hydrochloride 0.5% results in faster onset of sensory and motor blockade compared to levobupivacaine hydrochloride 0.5% alone. Duration of sensory and motor blockade and duration of analgesia were significantly prolonged when dexmedetomidine (75 μg) was added as an adjuvant to levobupivacaine hydrochloride 0.5%. Dexmedetomidine (75 μg) as an adjuvant to levobupivacaine hydrochloride 0.5% provides superior quality of analgesia (Lower VAS score) without any significant hemodynamic instability.



Graph 1: Mean heart rate (per minute)

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REVIEW ARTICLE

Hepatitis B Diagnosis in Blood Bank: Evaluation and Challenges

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ABSTRACT

Hepatitis B virus (HBV) presents a higher residual risk of transmission by transfusion than hepatitis C virus (HCV) or human immunodeficiency virus (HIV). While most infectious blood units are removed by screening for hepatitis B surface antigen (HBsAg), there is clear evidence that transmission by HBsAg-negative components occurs, in part, during the serologically negative window period, but more so during the late stages of infection.

To encourage voluntary blood donation should be the first step of prevention. To reduce the risk of transfusion-associated hepatitis B, test for anti-HBc immunoglobulin M may be included in routine screening of donors' blood, as it has been proved to be an excellent indicator of occult HBV during window period. However, awareness and education of donors regarding the modes of HBV transmission, a stringent one-to-one donor screening and increasing the voluntary donor base should also be implemented to minimize the rate of transfusion-associated hepatitis B.

Keywords: Hepatitis B, HBV, Diagnosis, HBsAg, Nucleic acid testing, Voluntary blood donation.

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INTRODUCTION

Two billion people have been infected with hepatitis B virus (HBV) worldwide and approximately 380 million (6%) are chronic carriers. A total of 4.5 millions new infections are reported annually. Hepatitis B virus kills 6.20 lac people each year. In highly epidemic regions, including Africa, the Amazon Basin, and central South East Asia, the prevalence is 8 to 15% in developing regions. Hepatitis B virus infection mostly occurs early in life with high risk of chronic liver disease. It can carry through blood and other body fluids, and is transmitted through mother to child, child to child and through sexual or parenteral contact. Vertical transmission is frequent in Asia. The rate of spontaneous HBV clearance is lower in children. Up to 90% of children infected during the first year of life and almost 50% of those infected between the age group 1 to 5 years will develop chronic hepatitis. A total of 25% of adults infected during childhood will die premature from liver cancer or cirrhosis. Hepatic cellular carcinoma (HCC) is the fifth most common malignancy worldwide and the third most common cancer in poor countries. Cirrhosis and HCC cause one million deaths each year mostly 80% in under-privileged regions. These regions face a lack of healthcare workers, poor medical infrastructures, insufficient screening, and poor access to care and treatment. At a time when morbidity and mortality of chronic liver disease has been widely improved in wealthy countries by new innovative strategies and potent antiviral drugs, it is now urgent to face the challenges of better management of chronic hepatitis in resource-poor countries from the perspectives of global health and social justice. And 2.4 million individuals have human immunodeficiency virus (HIV) and HBV coinfection. Hepatic cellular carcinoma is responsible for a quarter of liver-related deaths in HIV patients. Accurate data on prevalence, natural history, and severity of liver injuries in HIV-infected individuals living in resource-limited countries are lacking.

Resource-limited settings maintain the hepatitis epidemic due to many reasons:

Imperfect vaccination coverage—Although most countries vaccinate all children against HBV, it is not covering even 50% in Asia. Poverty and illiteracy, access to treatment for hepatitis B is very limited. The wide spread use of lamivudine rather than tenofovir as first-line treatment of HBV is worrisome due to alarming increase in drug resistance causing uncontrolled viral replication and hence cirrhosis and HCC. The high cost of antiviral therapies, the lack of medical infrastructure and laboratories, shortage of healthcare workers and diagnostic tools. Controlling HBV is very complex. In conclusion, access to screening, with improved diagnostic

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strategies, is of paramount importance for global health and social justice.

It is the responsibility of health policy makers, medical doctors, scientist, and governments to improve screening of HBV markers. The carrying out of cost-effective studies is urgently needed to demonstrate the burden of acute, chronic, and occult infections of HBV.[1]

The HBV is an enveloped deoxyribonucleic acid (DNA) virus that belongs to the family Hepadnaviridae and features partially double-stranded relaxed circular DNA. There are eight genotypes of HBV, classified from A to H, and these are distributed across different geographic zones. The entire genome is 3.2 kb in length and replicates by reverse transcription of an intermediate known as pregenomic RNA. The infectious viral particle is spherical, 40 to 45 nm in diameter. The virus consists of an inner nucleocapsid or core, surrounded by a lipid envelope containing virally encoded surface proteins. These protein markers are mainly used to screen the appearance of hepatitis infection with help of serological tests.

All three coat proteins of HBV contains HBsAg, which is highly immunogenic and induces anti-HBs (humoral immunity). Structural viral proteins induce specific T-lymphocytes, capable of eliminating HBV-infected cells (cytotoxic T-cells; cellular immunity). HBsAg is heterogeneous antigenically, with a common antigen designated a, and two pairs of mutually exclusive antigens, d and y, and w (including several subdeterminants) and r, resulting in four major subtypes: adw, ayw, adr, and ayr.

The distribution of subtypes varies geographically. Because of the common determinants, protection against one subtype appears to confer protection to the other subtypes, and no difference in clinical features has been related to subtypes.

In the United States, Northern Europe, Asia, and Oceania, the d determinant is common, but the y determinant is found at lower frequency. The d determinant to the near exclusion of y is found in Japan. The y determinant, and rarely d, is found in Africa and in Australian aborigines. y is also frequently found in India and around the Mediterranean. In Europe, the United States, Africa, India, Australia, and Oceania, the w determinant predominates. In Japan, China, and Southeast Asia, the r determinant predominates. Subtypes adw, ady, and adr are each found in extensive geographic regions of the world. Subtype ayr is rare in the world, but it is commonly found in small populations in Oceania. The c antigen (HBcAg) is present on the surface of core particles. HBcAg and core particles are not present in the blood in a free form but are found only as internal components of virus particles.[2,21]

The core antigen shares its sequences with the e antigen (HBeAg), identified as a soluble antigen, but no crossreactivity between the two proteins is observed.[2] Viral oligopeptides of 8 to 15 amino acids are loaded on host cell MHC-class I molecules and are transported to the surface of the cell. Hepatitis B virus-specific T-lymphocytes can then detect infected cells and destroy them. This cell deletion triggered by inflammation cells may result in acute hepatitis. When the infection is self-limited, immunity results. If HBV is not eliminated, a delicate balance between viral replication and immunodefence prevails, which may lead to chronic hepatitis and liver cirrhosis. In chronically infected cells, the HBV DNA may integrate into the host cell-DNA. As a long-term consequence, integration may lead to hepatocellular carcinoma.

Hepatitis B virus presents a higher residual risk of transmission by transfusion than hepatitis C virus (HCV) or HIV. While most infectious blood units are removed by screening for hepatitis B surface antigen (HBsAg), there is clear evidence that transmission by HBsAg-negative components occurs, in part, during the serologically negative window period, but more so during the late stages of infection. Screening of blood for detection of surface antigen, however, doesn't rule out the risk of transmission of hepatitis B totally, because during serological response of the host to infection, there is a phase during which the HBsAg cannot (avoid contractions in text) be detected in the blood, although hepatitis B infection is present. This phase is called as the 'core window period'. During this window period, detection of the antibodies to the hepatitis B core antigen serves as a useful serological marker for hepatitis B.[3] Evaluating the usefulness of anti-HBc screening is critical, particularly for India and other countries that have high hepatitis B endemicity.[4] It is also a good indicator of occult HBV infection during 'core window' period. Blood collected from the individuals chronically infected with HBV in whom HBsAg is not detectable and from donors with acute hepatitis who are in window period following disappearance of HBsAg and prior to the appearance of anti-HBs can effectively be removed from the inventory. Therefore, some blood banks from India incorporate anti-HBc testing in their donor screening protocol.[5] Routine anti-HBc screening of individual blood donations and nucleic acid amplification testing by pooling of sera is done in some countries to exclude these donations.[6]

Therefore, it is necessary to introduce the most advance techniques to identify the hepatitis DNA markers in the blood donor screening. The study of two automated nucleic acid amplification systems for blood donor screening shows that the Cobas 201 test was more sensitive than the Prolix Ulterior test.[7]

EVOLUTION IN DIAGNOSIS OF HEPATITIS B

The history of modern research on viral hepatitis began in the year 1963, when Nobel Prize winner Baruch S Blumberg (1925–2011) for the first time publicly reported the discovery of a new antigen named 'Australia antigen' (AuAg).

The earlier method of detection was to see the cytopathic effect of propagating virus in cell culture. Due to limitation of time taken by cytopathic effect, an alternate method was developed by observing viral material in electronic microscope. Another option was to observe hemagglutination reaction. These were the direct antigen detection methods.

The other approach was to detect antibodies produced in patient against HBV. The method most often used was 'compliment fixation reaction' (CFR), which requires four complex biological component mixtures from four different animals. Quantification of the CFR method was only possible by diluting the patient sera.

All attempts to identify the pathogen were unsuccessful for more than 8 years. The problem with viral hepatitis was so big that even human experimentation was done on the prisoners in 1950 in USA. In early days of evolution for blood donor screening, Blumberg used the Agar gel double diffusion, developed by Octerlony in the discovery of AuAg. The method was sensitive and specific as compared to the biochemical methods and technically simple than CFR.

In 1972, a team including biochemists Lacy Overby, Ghung-Mei Ling, and Richard Decker at Abbott Laboratories (North Chicago) developed a new testing principle for highly-sensitive detection of antigens or antibodies, the solid-phase sandwich radioimmunoassay named—Ausria-125. Antibodies coupled with fluorescent molecules which is measurable under UV light were specially used in viral detection. The fluorescently marked animal antibodies against human antibodies could be used to determine whether a person's serum contain the antibodies of hepatitis B antigen.

The new aspect was to use unlabeled antibodies by simple absorption to a surface (solid phase) and then allow person's serum to react with antibodies bound on special solid surface.

The specifically bonded protein complex could be detected by the antigen-specific antibodies labeled with iodine 125. The process opened new dimensions in detection sensitivity from several microgram/milliliter to nanogram/milliliter. The method was breakthrough for screening of blood donors and diagnosis.

In 1970, David Dane observed under electronic microscope that the Au antigen appears in the form of filamentous and spherical particles which were known as Dane's particles. Dane's particles on treatment with mild detergent produce core particles which induced the antibody response in human. This strongly suggested that the Dane particles were the actual virus causing hepatitis B. AuAg was obviously the surface antigen of the virus envelope and was named HBsAg (s for surface) thereafter.[8]

The introduction of Austria-125 was the beginning of an impressive development in virus diagnostics; however, the test had one major disadvantage: the radioactivity caused significant difficulties in the normal diagnostic laboratory.

It was, therefore, a big step forward when it became possible to label the antibodies used with enzymes and later with chemiluminescence-generating group. The assay of HBsAg was soon complemented by the detection of antibodies against HBsAg (anti-HBs) and HBcAg (anti-HBc).

Nucleic acid testing (NAT) is a molecular technique for screening blood donations to reduce the risk of transfusion-transmitted infections (TTIs) in the recipients, thus providing an additional layer of blood safety. It was introduced in the developed countries in the late 1990s and early 2000s and presently around 33 countries in the world have implemented NAT for HIV and around 27 countries for HBV. Nucleic acid testing technique is highly sensitive and specific for viral nucleic acids. It is based on amplification of targeted regions of viral ribonucleic acid or DNA and detects them earlier than the other screening methods, thus narrowing the window period of HIV, HBV, and HCV infections. Nucleic acid testing also adds the benefit of resolving false-reactive donations on serological methods which is very important for donor notification and counseling.

Serology assays are performed on individual samples, while NAT is performed on either the individual donation (ID) or on a wide array of minipool (MP)-NAT testing formats. The limit of detection of ID-NAT assays equals the analytical sensitivity and that of pool testing reflects the pool size-dependent decreased sensitivity also known as screening sensitivity. To many end users, it is not obvious that pool testing will have less sensitivity. In this review, mathematically predicted pool size-dependent increase in risk days which is applicable to assays of all technologies is substantiated with published experimental results with NAT standards, clinical NAT only detected yields and detection misses by MP-NAT. In the second half, the blood banking system in India, the donor base, and the variables in serology testing are discussed to explain the wide range of reported NAT yields at 1/300 to 1/17,753. Currently, NAT is not mandated in India and the cost-benefit value of NAT

is being seriously debated.[9] The other issue of debate is whether the protocol used for NAT should be of mini-pool method or of individual donor NAT. For nucleic acid testing of blood donations, either the blood samples can be pooled together in a batch of six or eight prior to testing [mini-pool-NAT (MP-NAT)] or the tests can be run on every individual sample [individual donor-NAT (ID-NAT)]. It is been debated that pooling of samples results in decreased sensitivity of detection as the volume of individual samples gets decreased in pool. The study showed that higher viral load samples were detected even in diluted samples, but the viral load below 20 IU/ml was missed by MP-NAT only due to dilution factor.[10,11]

CHALLENGES IN DIAGNOSIS OF HEPATITIS B IN BLOOD DONORS

Many confirmatory tests often used include western blot assays, line immunoassays, recombinant immunoblot assays, indirect fluorescent antibody assays, and enzyme-linked immunosorbent assays (ELISAs).

Each assay performance depends on the following basic parameters:

Sensitivity: Is a measure of the ability of the assay to identify correctly sera that contain surface antigen to HBV (reference assays positive). Thus, sensitivity is the number of true positive sera recognized by the assay under evaluation as positive (a), divided by the number of sera identified by the reference assays as positive (a + c), expressed as a percentage.

Specificity: Is a measure of the ability of the assay to identify correctly sera that do not contain surface antigen to HBV (reference assays negative). Thus, specificity is the number of true negative sera recognized by the assay under evaluation as negative (d), divided by the number of sera identified by the reference assays as negative (b + d), expressed as a percentage.[12]

HBsAg rapid diagnostic test (RDT) kits and other HBV rapid tests (HBsAb rapid tests, HBeAb rapid tests, HBeAg rapid tests, HBcAb rapid tests) are also referred to as one-step HBV testing kits. HBV testing kits are based on the principle of sandwich immunoassay for determination of HBsAg, HBsAb, HBeAg, HBeAb, and HBcAb in human serum or whole blood samples. Hepatitis B rapid test kits are widely used as an aid in the screening diagnostic test of hepatitis B infection.

The earliest HBV serological marker is its surface antigen HBsAg, but others develop in the course of the infection. Thus, one main target in HBV screening includes serology of HBsAg; however, routine screening for its core antibody (anti-HBc) is not recommended because of nonspecific results.[13] Most RDTs for HBV are based on agglutination or lateral-flow principles and qualitatively detect the presence of HBsAg. These are also available for detecting other HBV serological markers, such as the HBeAg and antibodies to HBs, HBc, and HBe. Various improvements are being made to these lateral flow assays for HBV. As declared in one analysis in 2008,[14] 'the major challenge for HBsAg rapid tests is to detect the low levels of the target antigen that are present in a relatively high proportion of asymptomatic blood donors in order to achieve a clinical sensitivity similar to that of enzyme immune-assays (EIAs)'. With the wide genetic diversity in HBV (genotypes A-H), there is high antigenic variation of HBsAg and a need for high antigen concentrations of some variants in order for them to be detected with commercial kits. An evaluation of various HBsAg assays using panels from the International Consortium on Blood Safety (ICBS) showed low analytical sensitivities of RDTs for HBsAg detection, as not detecting International Consortium on Blood Safety dilutions as positive, despite having high specificities.[15] Thus, sensitivity remains an issue for current HBV RDTs (HBsAg), despite excellent specificity. This emphasizes the continuous quest to improve the sensitivity of RDTs, which is indispensable for blood safety. An example of such an improvement is the new HBsAg rapid immunochromatographic assay based on the signal amplification system, which has been evaluated and shown to have enhanced sensitivity.[16]

The confirmation of RDT results is of relevance for issuing accurate results to blood donors, as well as for purposes of acquiring accurate epidemiological data; for blood safety, sensitivity is of the utmost importance. Wherever no established quality systems exist in resource limiting settings, confirmation is not necessary, because all reactive blood units should always be discarded. However, in settings with an established quality system, the World Health Organization recommends repeat testing, in duplicate, of the same sample and with the same assay before conclusions are drawn.[14] Furthermore, repeat testing may be performed with an alternative assay, either an RDT or an EIA.[17,18]

An individual positive for HBsAg is considered to be infected with HBV and is, therefore, potentially infectious. Confirmation of a reactive HBsAg ELISA screening test is usually done by performing a neutralization test using a specific anti-HBs antiserum in the same screening ELISA. Other HBV markers which can be used diagnostically include—HBeAg, immunoglobulin M (IgM) anti-HBc, total anti-HBc, anti-HBe, and anti-HBs. The presence of HBeAg indicates that an individual is of higher infectivity and seroconversion to anti-HBe correlates with reduced infectivity. An acute infection suggests that the infected

person is progressing toward resolving their infection. Individuals who have seroconvert from HBsAg to anti-HBs have resolved their infection and are immune to further HBV infection.

The study was conducted by the ICBS to identify high-quality test kits for detection of HBV surface antigen (HBsAg) for the benefit of developing countries. A total of 70 HBsAg test kits from around the world were evaluated comparatively for their clinical sensitivity, analytical sensitivity, sensitivity to HBV genotypes and HBsAg subtypes, and specificity using 394 (146 clinical, 48 analytical, and 200 negative) ICBS master panel members of diverse geographical origin comprising the major HBV genotypes A to F and the HBsAg subtypes adw,[2,4] adr, and ayw 1 to 4. The results of the performance evaluation of 70 HBsAg test kits showed that the diagnostic efficacy of the tests differed significantly. The sensitivity range between the most sensitive HBsAg devices and the least sensitive HBsAg assays was more than 300-fold. Enzyme immunoassays, in general, performed better than rapid assays. However, also within the EIAs there was a significant 200-fold variation in sensitivity; moreover, five EIAs were less sensitive than rapid assays. A relatively high number of assays, including all rapid tests, were of poor sensitivity rendering them unsuitable for HBsAg detection at low concentrations. Therefore, these assays cannot be recommended for use within a public health context, for example, blood screening. Genetic variability in the S gene additionally impaired diagnostic efficacy.[16]

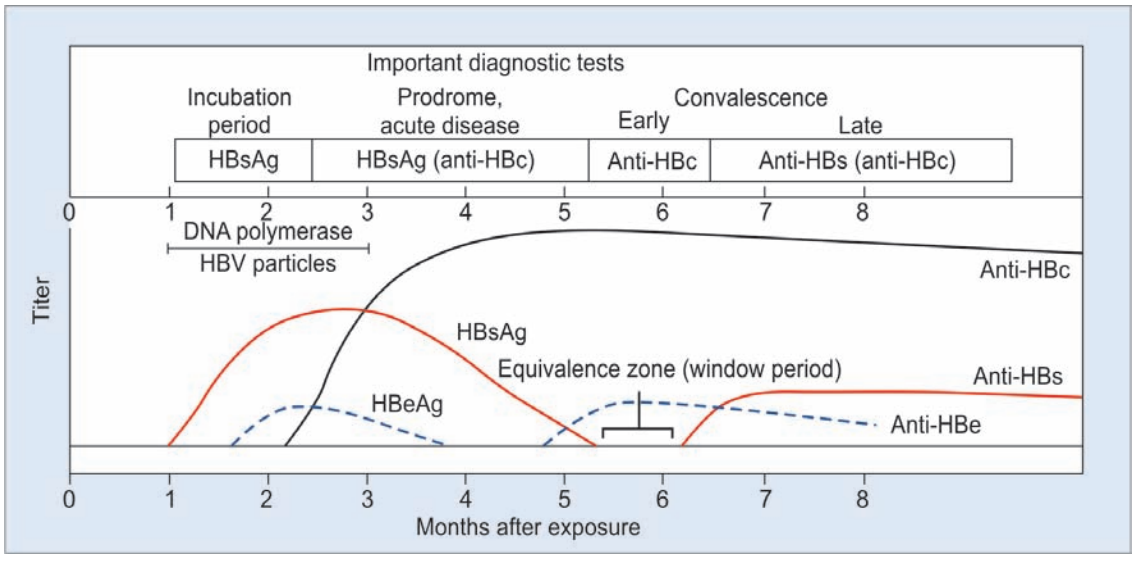
Diagrammatic representation of serology of hepatitis B infection and diagnostic test to be used are shown in Graph 1.

In the following conditions, HBsAg–negative individuals may have undetectable circulating viruses present in the blood:

1. Carriers with HBsAg below the detection level can transmit HBV by blood transfusion.
2. Subjects infected with HBV may show HBsAg negative result owing to point mutation in the precore region of the virus, resulting in inability to synthesize HBsAg. Fulminant hepatitis developed in recipients of HBsAg-negative blood from such donors infected with mutated virus. In all those donors, high levels of anti-HBc were present.
3. In acute infection, there are two periods when HBsAg may be undetectable although the subject can transmit HBV–during early incubation period when both HBsAg and anti-HBc are undetectable, and after clearance of HBsAg but before anti-HBs has become detectable (diagnostic window). In this phase, anti-HBc and anti-HBe can be detected.[19]

In India, blood screening for HBV, HIV, and HCV is done by serological tests for HBsAg and antibodies to HIV-1/2 and HCV. The screened seronegative donations are still at risk for TTIs, and thus need for a sensitive screening test arises to decrease this residual risk which has been reduced significantly over the last 2 to 3 decades in western countries, where NAT has been implemented. Nucleic acid testing has been started in few centers in India, but it is not a mandatory screening test for TTIs as per Drug and Cosmetics Act, 1940 and the rules therein.[20] Major barriers in implementing routine NAT testing in India are its high cost and lack of technical expertize in most of the blood centers.

Donations negative for HBsAg, but positive for HBV DNA, with or without the presence of HBV antibodies, correspond to 'occult' HBV infection (OBI). The frequency of OBI depends on the relative sensitivity of both HBsAg and HBV DNA assays. It also depends on the prevalence



Graph 1: The serological pattern of hepatitis B virus and helpful diagnosis methods

of HBV infection in the population. Occult HBV infection may follow recovery from infection, displaying antibody to hepatitis B surface antigen (anti-HBs), and persistent low-level viremia, escape mutants undetected by the HBsAg assays, or healthy carriage with antibodies to hepatitis B e antigen (anti-HBe) and to hepatitis B core antigen (anti-HBc). Over time, in the latter situation, anti-HBe and, later, anti-HBc may become undetectable. The critical question is whether or not OBI is infectious by transfusion. All forms have been shown to be infectious in immunocompromised individuals, such as organ- or bone marrow-transplant recipients. In immunocompetent recipients, there is no evidence that anti-HBs-containing components (even at low titer) are infectious. Anti-HBc only, with HBV DNA, can be associated with infectivity, as can rare cases of HBV DNA without any serological HBV marker. If HBV nucleic acid amplification technology is considered, the OBI viral load would usually be <500 IU/ml, making testing of plasma pools unsuitable unless the sensitivity of NAT significantly increases by genome enrichment or test improvement. It is recorded that the healthy blood donors which carry $<10^6$ IU/ml HBV DNA are positive for HBsAg, anti-HBs, and anti-Hbe. But the donors which carry infection with <1000 IU/ml of DNA are surely negative for all serological tests. Therefore, it is suitable to use an assay of highest sensitivity and specificity with detection limit as low as <10 IU/ml and <0.1ng/ml for HBsAg.[21]

Anti-HBc IgM is a useful marker during the 'core window', a short period in resolving acute HBV infection between the loss of serum HBsAg and the appearance of anti-HBs. Hepatitis B virus DNA is the earliest detectable marker in acute HBV infection. Hepatitis B virus DNA testing is particularly useful in the detection of the early phase of acute HBV infection prior to the appearance of serum HBsAg; for this reason HBV DNA is tested using nucleic acid amplification technology in blood and blood products in resource-rich countries. The appearance of anti-HBe followed by the appearance of anti-HBs is a characteristic of acute resolving HBV infection. The anti-HBs response remains detectable for several years following recovery from acute HBV infection and it indicates protective immunity. Anti-HBc IgG persists for several decades, if not for life, following acute HBV infection. In areas of low HBV endemicity, anti-HBc screening of blood and blood products in addition to HBsAg testing is performed to identify past exposure to HBV.[22]

The several molecular mechanisms involved in occult hepatitis B infections include mutation and deletion of HBV genome, treatment-associated mutations, coinfection with other markers, host immune response, epigenetic changes, and genetic integration.[22]

Hepatitis B virus precore G1896A mutation is associated with HBeAg seroconversion. This mutation and the adjacent G1899A mutation also appear to be associated with increased risk of hepatocellular carcinoma. Appearance of G1896A or G1899A mutation in the precore region is correlated with increased risk of HCC.[23,24]

The study among the blood donors of Odisha (Behram, Ganjam) found a stop codon in HBV/D2 at 69th position amino acid which results in a truncated HBsAg gene, lacking the total 'a' determinant region, which might be one reason for HBsAg negativity making the gene non-functional.[25] This mutation has also been documented in subgenotype D1.[26]

CONCLUSION

The use of recombinant multiepitop protein over expressed in *Escheria coli* can be used in diagnostic kit for hepatitis B, with several advantages: lower cost, facilitated manipulations, and elimination of problems concerning concentration of different peptides in the kits, but still it is not determined.[27] To encourage voluntary blood donation should be the first step of prevention. To reduce the risk of transfusion-associated hepatitis B, test for anti-HBc IgM may be included in routine screening of donors' blood as it has been proved to be an excellent indicator of occult HBV during window period. However, awareness and education of donors regarding the modes of HBV transmission, a stringent one-to-one donor screening and increasing the voluntary donor base should also be implemented to minimize the rate of transfusion-associated hepatitis B.[19]

Even single-sample HBV NAT may not substitute for anti-HBc screening, as indicated by studies of donors with isolated anti-HBc who have extremely low DNA levels undetectable by standard single-sample NAT and who have been associated with transfusion-transmitted HBV. Moreover, HBsAg testing may still be needed even in the setting of combined anti-HBc and NAT screening. HBsAg-positive units from donors in the chronic stage of infection may contain very low or intermittently detectable DNA levels that single-sample NAT would miss. Although such donors are usually anti-HBc reactive and would be interdicted by anti-HBc screening, some lack anti-HBc. Extensive parallel testing will be needed to determine whether single-sample NAT in combination with anti-HBc might be sufficient to detect all the infectious donors currently interdicted by HBsAg testing. In the future, the current fully automated HBsAg assays may incorporate significant sensitivity improvements and automated single-sample HBV NAT may become a reality. Each country will need to develop its blood screening strategy based on HBV endemicity, yields of

infectious units detected by different serologic/NAT screening methods, and cost-effectiveness of test methods in ensuring blood safety.[28]

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REVIEW ARTICLE

Association of Host and Pathogenic Variation with Sexual Transmission of HIV

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ABSTRACT

Human immunodeficiency virus (HIV) is genetically extremely variable due to the poor proof reading activity of its reverse transcriptase enzyme. Human immunodeficiency virus isolates are highly variable over time, and exhibit changes in biological phenotype during the course of infection. Different HIV variants exist in different tissues, cells and secretions; including genital secretions and cells of human males and females. Virus present in the urogenital cells and secretions determines the risk for sexual transmission of HIV. The precise association of viral variants from genital secretions and cells in the sexual transmission of HIV to the partner is not fully understood. The presence of viral variants may influence affinity to different host cell receptors which may affect the transmission, infectivity, cellular immunity and pathogenesis of HIV. Delineation of the role of host and pathogenic variation will lead to a better understanding of the process of sexual transmission of HIV. Furthermore, it will also help in designing the strategies for development of preventive or therapeutics vaccines and microbicides for control and management of HIV/AIDS.

Keywords: HIV, Host factors, Pathogenesis, Sexual transmission, Viral variants.

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INTRODUCTION

Pandemic of acquired immunodeficiency syndrome (AIDS) is increasing globally, and it is estimated that more than 39 million people, including adults and newborns, have died of AIDS. Human immunodeficiency virus (HIV) transmission often occurs at mucosal surfaces and sexual transmission of HIV, though inefficient, is the most prevalent route accounting for about 75 to 80% of infections.[52] Extreme genetic variability is the hallmark of HIV infection due to the poor proof reading activity of its reverse transcriptase enzyme. Human immunodeficiency virus isolates are highly variable over time. They exhibit changes in biological phenotype during the course of infection. Presence of different viral variants in different cells and secretions of the same individual may influence sexual transmission of HIV and viral affinity to different host cells which may affect transmission, infectivity, cellular immunity and pathogenesis of HIV. Virus present in the urogenital cells and secretions determines the risk for sexual transmission of HIV. The precise association of viral variants from genital secretions and cells in sexual transmission of HIV to partner is not fully understood. It is not only important to understand the events that occur during sexual transmission of this virus, but also the impact of host and pathogenic variation on HIV transmission. In this review article, we look at a number of factors relevant to the transmission of this infection; with particular emphasis on the challenges posed by host and pathogenic variation on the transmission of HIV. Insights into the process of sexual transmission of HIV will enable the rational design of prevention strategies, such as microbicides and vaccines.

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SEXUAL TRANSMISSION AND THE RISK OF HIV-1 INFECTION

Many aspects of HIV transmission are still unclear and are the focus of extensive research and investigation. An important question which still remains unanswered is whether the transmission of HIV is mediated mainly by the cell-free virus or by infected cells. Both the forms of this virus have been reported to be present in human genital secretions namely semen in the male and vaginal fluids in the female.[12] Moreover, the risk of male to female transmission of HIV is considered to be higher as compared to that of female to male.[8,45]

Viral load of the infected individual is also known to be a major determinant of the risk of HIV-1 transmission. A 10-fold increase in viral load has been reported to result in a 2.5-fold increase in the transmission of HIV-1 in serodiscordant couples.[20,46] Although the risk of sexual transmission of HIV has been reported to correlate with the amount of virus present in the blood of the source partner,[46] the correlation between the viral load in the

blood and genital compartments is inconsistent.[10,32,33] The viral load in genital fluids is quite variable, and is generally lower in untreated individuals than in the blood.

The clinical stage of infection in the transmitting partner is also known to be a key determinant for the efficiency of transmission, with the risk of infection from individuals with acute infection being higher than that in individuals with an established infection.[40,45,53]

The integrity of the epithelial barrier in the vagina of the female also appears to be a crucial determinant for HIV entry via the sexual route. An intact mucosal epithelium is impervious to infection by HIV. However, any micro-aberrations of the vaginal epithelium due to physical insults or inflammation are also known to increase the infectivity of the virus.[25,26]

Another important factor which impacts viral transmission is the presence of sexually transmitted diseases (STDs), particularly those that result in ulceration and genital inflammation. There is an increased HIV shedding into the genital tract and a concomitant increase in susceptibility to infection in such cases.[21] Sexually transmitted diseases, when accompanied by ulceration, result in breakdown of the mucosal barrier which makes the recipients more susceptible to infection. Additionally, there is also an increase in the number of susceptible cells in the mucosa through inflammation. Bacterial infection of the vagina in the female also results in enhanced infectivity due to an increase in the pH of the vaginal fluid. This is attributed to slower virus inactivation and more efficient env-mediated fusion at a higher pH.[21,12]

ASSOCIATION OF HOST CELLS WITH HIV TRANSMISSION AND PATHOGENESIS

Association of Genital Secretions with the Sexual Transmission of HIV

Human immunodeficiency virus-1 present in the seminal plasma, seminal leukocytes and sperm is known to be the primary source of infection.[38] Seminal plasma and leukocytes were considered to be the sole source of infection, but subsequent reports demonstrated that HIV binds and enters into the spermatozoa and further transmits the virus into distal cells. Moreover, due to the acidic pH of the vagina and the temporal sequel of seminal leukocytes in the vaginal tract, infection of female through cell-free virus or by seminal leukocytes from the male seems less risky. Furthermore, the viral load required for transmission of HIV through the vaginal route has been demonstrated to be very high as compared to that of the systemic route.[38] This suggests that the spermatozoa is a risk factor in sexual transmission of HIV. However, due to lack of conventional CD4 receptors on spermatozoa, the precise mechanism of sexual transmission of HIV has not been fully understood. Presence of human mannose receptor (hMR) on spermatozoa has been shown to be responsible for sexual transmission of HIV. Human immunodeficieny virus binds specifically to hMR and enters into the sperm which further transmits the virus into urogenital cells.[7,19] Therefore, the sperm-bound virus may determine the risk of sexual transmission of HIV.

Human Immunodeficiency Virus Binding Receptors Present on Different Host Cells

Human immunodeficiency virus binds to CD4 receptor, CXCR4 and CCR5 co-receptors as well as to other receptors on host cells which include hMR, dendritic-cell-specific ICAM-3-grabbing–non-integrin (DC SIGN) receptor, galactosyl ceramide (GalCer) receptor, heparan sulfate (HS) and syndecan-3. Sexual transmission of HIV has been shown to be associated with hMR as human sperm and vaginal epithelial cells are devoid of conventional CD4 receptors. Human immunodeficiency virus specifically binds to hMR and further transmits the virus into distal cells. Furthermore, it has also been reported that less than 10% vaginal epithelial cells in seronegative female partners of serodiscordant couples showed the presence of hMR, while 90 to 95% vaginal epithelial cells of the normal females from general population showed the presence of hMR.[29] The study suggested the role of hMR in the sexual transmission of HIV.

Dendritic cells (DCs) are also known to play a major role in HIV pathogenesis. Peripheral or surveillance mucosal DCs are distributed in the vaginal, ectocervical and anal mucosa, allowing contact with HIV during mucosal exposure.[17,39] Following vaginal entry of the virus, DCs have been shown to be responsible for further transmission,[27] and subsequently stimulated T-cells may also play a key role in establishing infection. Different types of DCs from skin, mucosa and blood of humans and macaques can participate in highly productive CD4-dependant and CD4-independent HIV and simian immunodeficiency virus infection. These host cell responses are also affected by viral variation and presence of distinct variants in different cells and secretions of the same individual.

Astrocytes are also found on CD4 negative cells in the brain and it has been postulated that HIV neuropathogenesis occurs via hMR. In human astrocytes, HIV binds to hMR and activates matrix metalloproteinases, which in turn are reported to degrade the extracellular matrix proteins.[36]

Association of Viral Variation in HIV Transmission and Pathogenesis

Poor proofreading activity of HIV-1 reverse transcriptase enzyme results in extensive diversification during the natural course of infection.[15] Peripheral blood mononuclear cell (PBMC)-derived isolates exhibit increased variability over time. They exhibit changes in the biological phenotype during the course of infection.[11] Human immunodeficiency virus-1 isolates from PBMCs during early infection show slow/low titer, non-syncytium inducing phenotype and preferentially infect monocyte-derived macrophages (MDM) and PBMCs. With the onset of AIDS, most but not all patients harbor rapid/high titer viruses, often with syncytium-inducing phenotype, which infect T-cell lines efficiently but may have reduced ability to infect MDM.[50,51] The genetic diversity of HIV-1 in an infected person, typically investigated in blood plasma, manifests itself as a collection of closely related but genetically distinct viral variants termed 'quasispecies'.[15] Distinct HIV variants have been shown to exist in different tissues and secretions including lymph nodes, spleen, brain, lungs and semen.[16] These variants also show changes in the biological phenotype during the course of infection. Furthermore, viral variants present in sperm and seminal secretions may also determine the risk of sexual transmission of HIV. The infectivity of HIV-1 is known to vary because of the differences in virus subtypes, and the set of virus quasispecies present in an infected individual.

Free as well as cell-associated virus has been detected in genital cells and secretions which are the major source of sexual transmission of HIV.[1,3,37] Male and female genital tract cells and secretions serve as sites of viral replication and are likely to differ from peripheral tissues in immunological surveillance, target cell characteristics and efficiencies of drug penetration.[14,22,23,30,31,44,47,49,55,56] Recent studies in sub-Saharan Africa with subtype B and subtype C transmission pairs have suggested that a single variant from the chronically infected partner can establish infection in their newly infected partner. However, in subtype A, infected individuals from a sex worker cohort and subtype B individuals from STD clinics demonstrated that the infection was frequently established by multiple variants too.[24]

Human immunodeficiency virus-related mortality and morbidity has been significantly reduced by highly active antiretroviral therapy (HAART). However, lack of proofreading activity of HIV reverse transcriptase results into continuous mutations during the course of infection, which leads to drug resistant mutations, and may therefore accelerate the viral load in circulation. Human immunodeficiency virus present in genital tract secretions has been reported to be responsible for sexual transmission of HIV. Presence of drug resistant viral mutant has been reported in genital tract secretions due to poor accessibility to different antiretroviral drugs[34,43] and may also influence HIV transmission to the partner.

Subtype C has been reported in nearly every region affected by HIV and predominates in India and Africa. However, little is known about sequence variation of HIV-1C in India. Lole et al[35] studied the complete genome of HIV-1 isolates of six seroconverters from Pune, India. HIV-1C isolates from these individuals were amplified, cloned and sequenced. Five out of six were reported to be subtype C, while one was a mosaic of subtype A and C with multiple break points in env, negative factor and long terminal repeats (LTR). A total of 38% well defined cytotoxic T-lymphocyte (CTL) epitopes were identical and showed substantial differences with CTL epitopes of subtype B.

Genotypic characterization of C2-V3 region of HIV-1C from PBMCs, spermatozoa and vaginal epithelial and cervical cells of the same individual demonstrated the presence of distinct viral variants in PBMCs and sperm. Translated amino acid sequences of C2-V3 region of these isolates present in PBMCs and sperm[28] or vaginal epithelial cells, and cervical cells of the same individuals showed different numbers of N-linked glycosylation (NLG) sites; suggesting the differential affinity of these variants to host cells. Moreover, these variants showed differential infectivity in PBMCs and sperm, or vaginal epithelial and cervical cells of HIV-infected individuals.

The biological determinants that influence the transmissibility of different viral variants within the genital tract of the HIV-infected source are still incompletely understood. Since transmitted virus represents the initial virus that the immune system encounters, the understanding of its composition will be critical in development of modalities in prevention of sexual transmission of HIV. The HIV variants in urogenital cells may also influence the response to anti-retroviral therapy (ARV) drugs. Therefore, the drug resistant mutations of the HIV variants, PBMCs, as well as urogenital cells and secretions will be useful for administration of appropriate combination of ARV drugs to control the disease.

DISCUSSION

Spermatozoa are known to be a risk factor for sexual transmission of HIV.[4,9,41-42,48] Sperm-associated virus has also been shown to be efficiently transmitted to DCs, macrophages and T-cells even at lower vaginal pH. Furthermore, HIV transmission also results in the phenotypic maturation of DCs and the production of interleukin-10 (IL-10) but not interleukin-12 (IL-12), suggesting the role of sperm associated virus in mucosal transmission.

Cell-free virus as well as proviral DNA has been detected in the sperm[1,3,4] and HIV replicates in the sperm mitochondria.[5] Presence of distinct viral variants has not only been detected in PBMCs,[6,11,13,44] but also in genital secretions and tissues during the course of infection.[56,57] Human immunodeficiency virus-1 infected men receiving highly active antiretroviral therapy have been reported to have undetectable levels of viral ribonucleic acid (RNA) in blood plasma, but showed the presence of virus in seminal cells.[2] The present study also demonstrated that one of the studied participants on ART had undetectable viral load in the blood plasma, but seminal plasma showed the presence of 457 copies of viral RNA. In addition, five out of the seven sperm samples from infected males were found to be infectious as detected by estimation of p24 antigen levels in the culture supernatant following co-culture with PBMCs from normal individual. The viral load in seminal plasma of one of the individuals was undetectable, but showed the presence of proviral DNA in the spermatozoa. Therefore, sperm washing procedure used for assisted-reproductive technology may not always prevent HIV transmission to fetus and/or female partner. This also confirms the earlier findings that sperm-associated HIV is the risk factor for sexual transmission of HIV, and although the seminal viral load is undetectable the sperm may still carry the virus.

The characterization of viral variants by sequence analysis of C2-V3 region of HIV-1C env gene demonstrated the presence of distinct variants in the spermatozoa and PBMCs of the same individuals (Fig. 1). Figure 2 shows the comparative translated amino acid sequences of C2-V3 region of HIV-1C isolated from PBMCs of 12 infected males, which demonstrated variation in the sequence of all the participants studied. Figure 3 shows the comparative translated amino acid sequences of C2-V3 region of env gene of HIV-1C from sperm samples. The constant regions have been highlighted in green color. The region from 241 to 263 predominantly showed the conserved region in all individuals and corresponds to the C2 region of env gene.[28] This may possibly be an important region for consideration of a potential candidate for development of peptide-based vaccine. Additionally, smaller constant regions were also found to be conserved in C2 as well as the V3 regions. Association of these constant and variable regions needs to be investigated in a larger population to determine their role in transmission and response to neutralizing antibody.

The viral variants in sperm and PBMCs also show a variable number of NLG sites. Alterations in NLG patterns have been reported to successfully mask effective antibody-neutralization responses.[54] Furthermore, an N-glycan within the HIV type 1 gp120 V3 loop has been reported to affect virus neutralization.[57] Numbers of NLG sites also showed variability. Moreover, variable numbers of NLG sites have also been observed in the sperm and PBMCs of the same individuals.

The sperm as well as PBMCs of two individuals showed absence of NLG sites in the V3 loop. The absence of NLG sites in the V3 loop was also observed in PBMCs of additional six individuals suggesting possible association of these variants with CXCR4 co-receptor usage in these males. Remaining nine individuals showed the presence of NLG sites in the V3 loop of HIV-1C env gene, which indicates CCR5 co-receptor usage. The numbers of NLG sites in the sperm-associated virus were found to be less in four out of six individuals as compared to those from PBMCs of the same individuals. Different individuals showed different number of NLG sites in C2-V3 region, suggesting the variable affinity of HIV for binding to

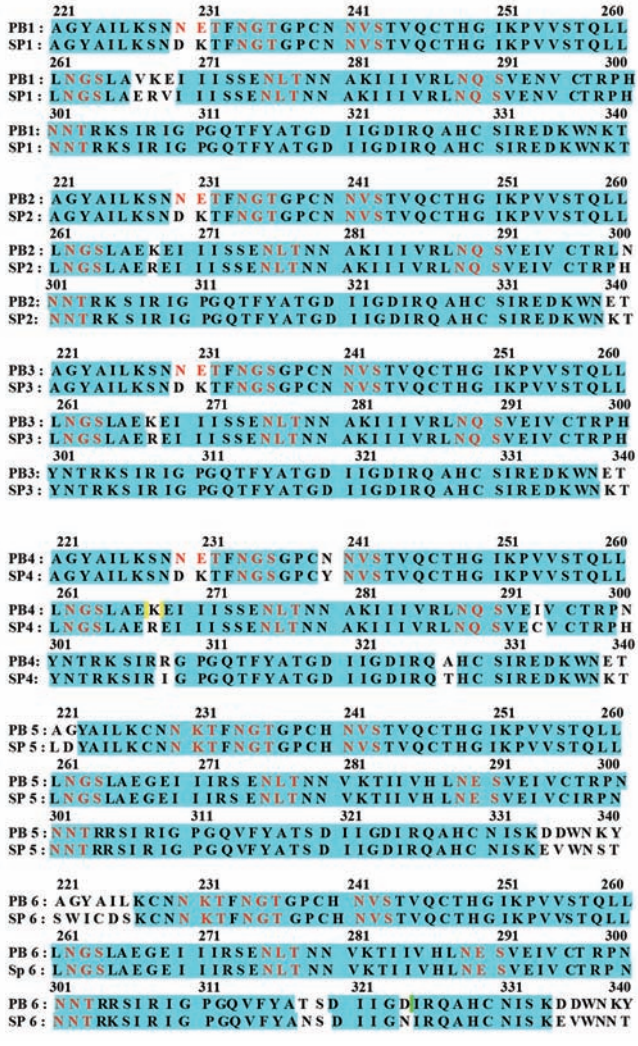

Fig. 1: Translated amino acid sequence of C2-V3 region of env gene of HIV-1C present in spermatozoa and PBMCs of the same individual. The letters highlighted with green color show the conserved regions of the variants in sperm (SP) and PBMCs (PB) of the same individual. Letters marked with red color are the NLG sites. C2 region 221 to 295; V3 region 296 to 340

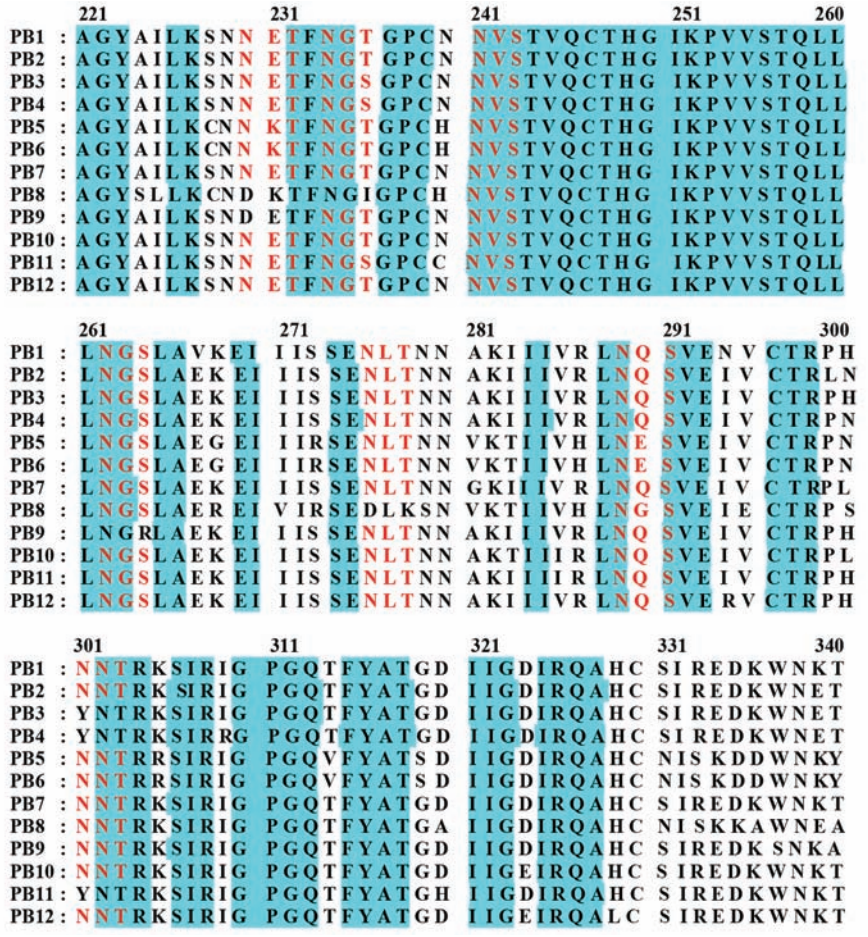

Fig. 2: Translated amino acid sequence of C2-V3 region of env gene of HIV-1C present in PBMCs of the infected individuals. The letters highlighted with green color show the conserved region. Letters marked with red color are the NLG sites. C2 region 221 to 295; V3 region 296 to 340

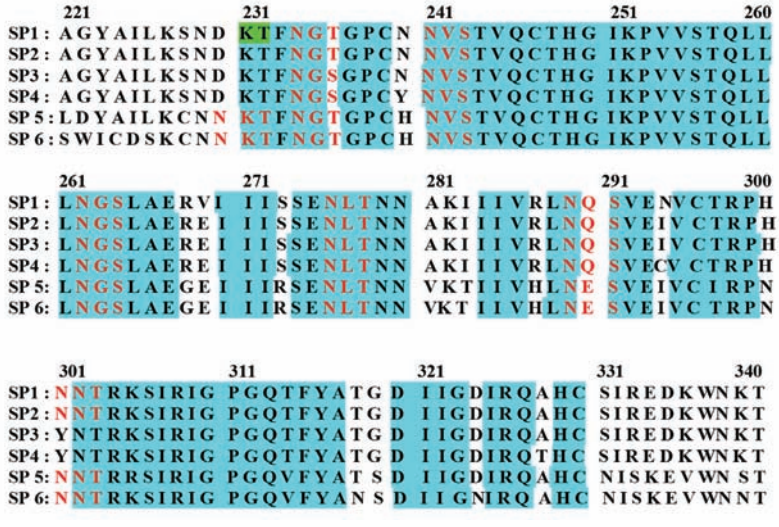

Fig. 3: Translated amino acid sequence of C2-V3 region of env gene of HIV-1C present in spermatozoa of the infected individuals. The letters highlighted with green color show the conserved region. Letters marked with red color are the NLG sites. C2 region 221 to 295; V3 region 296 to 340

different host cells of the infected individuals.[28] Four NLG sites were found to be conserved in all the sperm as well as PBMC samples. The number of NLG sites of viral envelope proteins, through the formation of a 'glycan shield', is one of the major mechanisms for blocking or minimizing virus-neutralizing-antibody response.[54] The density of gp120 NLG sites has been considered to be a significant obstacle to the design of an effective vaccine and the elicitation of a humoral immune response. Human immunodeficiency virus is now known

to bind to hMR on spermatozoa[18] which are devoid of conventional CD4 receptors. Therefore, the alterations in the number of NLG sites of HIV env gene and its association with transmission to distal cells, needs to be further investigated.

These studies suggest that the sperm-associated virus is the risk factor for sexual transmission of HIV, and possibly also for the transmission from parent to child. They also suggest that assisted reproductive technology is not a completely safe procedure for maternal or fetal transmission of HIV. Human immunodeficiency virus-1C env sequence diversity appears to influence affinity to receptor/co-receptor and effective immune responses against the virus, thereby implying a link between strong immune selection and slower disease progression. Moreover, the diversity of sperm-associated virus may influence it's binding to hMR which is a CD4-independent receptor, it's further transmission into distal cells and therefore, also the sexual transmission of HIV. However, the precise involvement of viral variants and alteration in the number of NLG sites, their association with sexual transmission of HIV and disease progression needs to be investigated in a larger population. This will be useful in designing strategies for prevention of HIV infection and also the development of therapeutics for HIV/AIDS.

CONCLUSION

Human immunodeficiency virus is primarily transmitted through the sexual route. Efforts are being made to understand the mechanism of HIV transmission, pathogenesis and control of HIV/AIDS. Recently, the presence of hMR has been demonstrated to be responsible for sexual transmission of HIV. Human immunodeficiency virus binds specifically to hMR on sperm and vaginal epithelial cells, and further transmits the virus into distal cells. Additionally, other receptors, such as DC-SIGN, GalCer, heparan sulfate and syndecan-3 have also been identified on different cells; and viral affinity to these receptors may influence HIV pathogenicity. However, the poor proofreading activity of HIV reverse transcriptase enzyme results into the presence of distinct variants in different cells and secretions of the same individual. These viral variants may influence HIV affinity to different host cell receptors, and therefore influence the pathogenicity and progression to disease, which remains the major challenge in management and control of HIV/AIDS. Therefore, the characterization of HIV variants and their affinity to different host cell receptors may provide the information to design the strategies for ART, prevention of sexual transmission of HIV and development of effective therapeutic and/or preventive vaccine.

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REVIEW ARTICLE

Pain and Joy in Implementation of Curriculum Reform: The University of Hong Kong Medical Faculty Experience

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ABSTRACT

The University of Hong Kong (HKU) Li Ka Shing faculty of medicine (established 1886), introduced curriculum reform in 1997, and implemented problem-based learning (PBL) as a part of hybrid curriculum. The reform made significant modifications to time-tabling including reorganization of basic sciences program into system-based blocks structured around PBL tutorials, lectures, practicals, demonstrations and relevant anatomy dissections. Assessment was also integrated at the faculty rather than departmental-based for the first three medical years.

During the reform, apprehension and concern in relation to outcomes and quality of graduates were raised, particularly on students' basic science foundation and whether students would be able to cope with demands related to PBL. To address these concerns, a study was undertaken to evaluate new graduates' performance from two aspects: (1) knowledge-based performance before their internship, and (2) on-the-job performance during their internship, under the old and new curriculum.

To evaluate intern's knowledge-based performance, a written test consisting of multiple choice questions and short answer questions, based on combination of basic sciences knowledge and clinical scenarios was given to two cohorts of old (2000–2001) and new (2002–2003) graduates. To evaluate graduate's on-the-job performance, scores from internship performance over the past 9 years were retrieved from the faculty. Results from the first 2 years of new curriculum graduates and the last two cohorts of old curriculum graduates demonstrated that they had similar basic sciences knowledge-based performance. On the other hand, new curriculum graduates did significantly better in their on-the-job internship performance. Areas of strength within our graduates were attitude to staff, sense of responsibility and attitude to patients.

Keywords: Curriculum reform, Problem-based learning, University of Hong Kong, Medical education.

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INTRODUCTION

A graduation dinner organized by the medical students of faculty of medicine, The University of Hong Kong (HKU) on 27 June 2002 had a special significance. It was the first batch of the Bachelor of Medicine and Bachelor of Surgery (MBBS) graduates, who joined new medical curriculum in 1997. A remarkable evening—reflective, enjoyable, and above all, filled with an air of certain sense of achievement for both students and faculty. Students recalled their pioneer spirits undergoing problem-based learning (PBL) tutorials, special study modules, continuous assessments, evaluation and also apprehension about the outcomes.

Students, who joined the new curriculum had put their trust into the faculty's wisdom in changing course structure and innovative learning methods. The journey from implementation to consolidation of the new and innovative curriculum was of course full of challenges, with pain and joy.

The stimulus for reform originated from a document 'Tomorrow's doctor', published by General Medical Council of United Kingdom.[1] A review undertaken at the medical faculty of HKU in 1993 also noted:

- Teaching was compartmentalized with no integration
- Students were too passive
- Curriculum was overcrowded
- There was lack of emphasis on primary healthcare issues
- Teachers taught more than necessary
- There was too much emphasis on big bang examinations
- The curriculum was too rigid and didactic
- Student self-learning was not encouraged.

Once the faculty approved implantation of reforms, dean of the faculty made 'failure is not an option' as a slogan for implementation; and reform process played out a real-life drama of proposals, counter proposals, deliberation, skepticism, disappointment, compromise, excitement, planning, implementation, hope and, above all, determination to succeed.[2]

Facing the Challenges

Staff Development Programs

Staff development workshop under auspices of Medical Education Unit [now Institute of Medical and Health Sciences Education (IMHSE)] were organized and run in a seamless manner; to train all basic sciences and clinical teachers in curriculum planning PBL tutorship, PBL case-writing, scenario-based exam questions, objective structured clinical education (OSCE), integrated lectures, simulated communication and clinical skills, academic counseling and effective continuous assessment, etc.

Curriculum Change to Integrated System Blocks and Clerkships

Old curriculum containing preclinical, paraclinical and clinical syllabi was changed to integrated system blocks. This change needed collaboration and participation of all basic and clinical departments working together to shortlist essential lectures, PBL tutorials and practicals, ultimately resulting in reduction of large number of lectures (Table 1).

Problem-based Learning

Concept of problem-based learning tutorials was fairly unknown and challenging to many staff members, particularly to basic science teachers who had to facilitate tutorials based on case scenarios. On other hand, clinical staff erroneously felt that they already 'teach' by PBL method, not realizing that the process of PBL meant to direct students to 'active learning'; not teacher-centered passive learning. Massive efforts were put into action in case-writing and PBL tutor workshops to highlight the process of facilitation in PBL. Nearly, 200 teachers became certified PBL tutors over the period of 6 months. In addition, four PBL cases were written for each system blocks to bring about integration of anatomy, physiology, biochemistry, pharmacology, pathology, microbiology and clinical sciences.

Physical Facilities

Faculty now has a 'state of art' faculty of medicine building, inaugurated in 2002, with academic, administrative and research blocks, all in one complex. Academic block provides a generous space to purpose-built PBL tutorial

Table 1: Integrated system-based curriculum structure

Year 1							
September		*January*				*May*	*June*
Introduction to health and disease block		Formative exam	System-based course			First exam	SSM
			Respiratory system	Cardiovascular system	Gastrointestinal system		
Year 2							
September						*May*	*June*
System-based course						Second exam	SSM
Urogenital system	Musculoskeletal system	Central nervous system	Head and neck system	Hematology/ immunology system	*Endocrine system*		
Year 3							
Late-August	*Mid-October*	*January*	*Mid-March*			*May*	*June*
Integrated block	Formative exam	Junior clerkship				Third exam	SSM
		Medicine-related block	Surgery-related block	Multidisciplinary block			
Year 4							
Mid-July			*January*				*June*
Senior clerkship			Specialty clerkship				
General medicine	Surgery	Multidisciplinary	Medicine	Obstetrics and gynecology			Pediatrics
Year 5							
Mid-July			*January*	*March*		*April*	*June*
Specialty clerkship			Revision	Final examination		SSM	Pre-internship program
Psychiatry	Surgery	F med/orth Sur/private					

rooms, clinical and virtual reality skills laboratories for medical and nursing students, computer assisted learning laboratory with nearly 50 internet ports, a conference center, seminar rooms, teleconference facility, exhibition hall, student amenities and a modern library. The facility, funded by Hong Kong jockey club, University of Hong Kong and many alumni and friends of the faculty has truly provided a conducive and excellent environment to complement learner-centered education philosophy.

Outcome Evaluation

There was some apprehension and concern in relation to outcomes and quality of graduates, who would undergo these reforms, particularly basic science foundation that students would have achieved through reduced number of didactic lectures to around 60% and selective practicals. Opinions were also expressed that new graduates may be unable to cope with demands related to PBL. To address these concerns, a study was undertaken to evaluate graduates' performance from two aspects: (1) knowledge-based performance before the internship, and (2) on-the-job performance during their internship, under the old and new curriculum.

Evaluation of Graduates' Performance before the Internship

Evaluation of students' performance was carried out during the pre-internship program of 3 weeks held in the month of June for all graduates after their final examination and just before the beginning of 1 year internship. A written test consisting of multiple choice questions (MCQs) and short answer questions, based on combination of basic sciences knowledge and clinical scenarios, was given to two cohorts of old and two cohorts of new graduates during their pre-internship program (Table 2).

Random samples of 60 MCQs of 'one best answer type' were selected to test students' knowledge in anatomy, physiology, biochemistry, pathology, microbiology and pharmacology in clinical context related to various body systems. The objective was to document adequacy or deficiency, if any, of basic sciences knowledge in new curriculum graduates as compared to old curriculum graduates when they complete the MBBS course.

Table 2: Number of interns attending written test

Curriculum	*Years*	*Number of interns*
Old	2000	143
	2001	140
New	2002	135
	2003	142

Besides MCQs, a survey of 'practical tasks' which students had observed/assisted/performed during MBBS course; and challenging questions on prescription practice, inter-professional communication and evidence-based information were included in the second part of written test.

Evaluation of Graduates' Performance during the Internship

Evaluation of interns' performance related to their on-the-job activities was documented every 3 months by their supervisors from 13 major hospitals in Hong Kong. Their performance assessment was based on eleven domains, namely—professional knowledge, clinical skills, attitude to patients, attitude to staff, willingness to learn, organizational ability, clinical judgment, attendance at educational activities, use of medical language, communication skills and sense of responsibility. The performance in each domain was ranked individually as unacceptable (score 0), needing help and counseling (score 1), poor (score 2), average (score 3), good (score 4), and excellent (score 5).

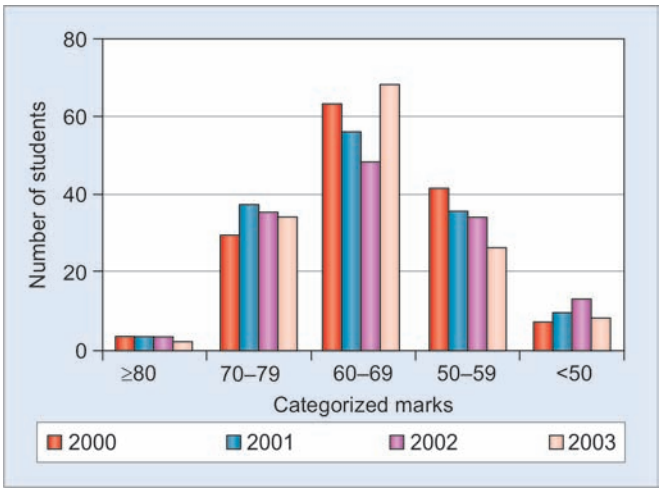
Mean scores in each domain from respective years of old curriculum interns (1999/2000–2001/2002) and new curriculum interns (2002/2003–2008/2009) were analyzed for comparison. Statistical analyses were performed using the statistical package for the social sciences (SPSS) computer program. We compared the mean scores of performance between the old and new curriculum using t-tests with significance level set at a p-value < 0.05. Effect sizes were determined to show the magnitude of the differences between different curricula for each analysis. Cohen stated that a strong effect size is 0.80, a moderate effect size is 0.50 and a weak effect size is 0.25.[3] Analysis of variance (ANOVA) was employed to evaluate the change in mean scores of performance over a period time. Objective of this exercise was to validate the perception, that clinical performance and other professional attributes of the new curriculum graduates should be better due to early clinical exposure and PBL structure with the integrated curriculum. In addition, we tried to identify the areas of strength and weakness of our graduates during their on-the-job performance.

RESULTS

Graduates' Performance Just Before the Internship

Scores derived from MCQ tests were organized into five groups of marks: < 50%, 50 to 59%, 60 to 69%, 70 to 79% and ≥ 80%; and were incorporated into the graph (Graph 1).

Statistical analyses of the cumulative scores of old and new curriculum graduates did not reveal any



Graph 1: Knowledge-based assessment during the preinternship program

significant difference. However, further review of scores of top 10 and bottom 10 students revealed, that some of the students from the first batch of new curriculum had relatively low scores as compared to other groups, mainly related to questions on microbiology and anatomy.

Besides the MCQ, survey of 'practical tasks' which students had observed/assisted/performed during MBBS course; and challenging questions on prescription practice, interprofessional communication and evidence-based information were included in the second part of written test. New curriculum graduates did better in these areas as they had opportunities to observe practical tasks earlier in year one and two along with PBL tutorials.

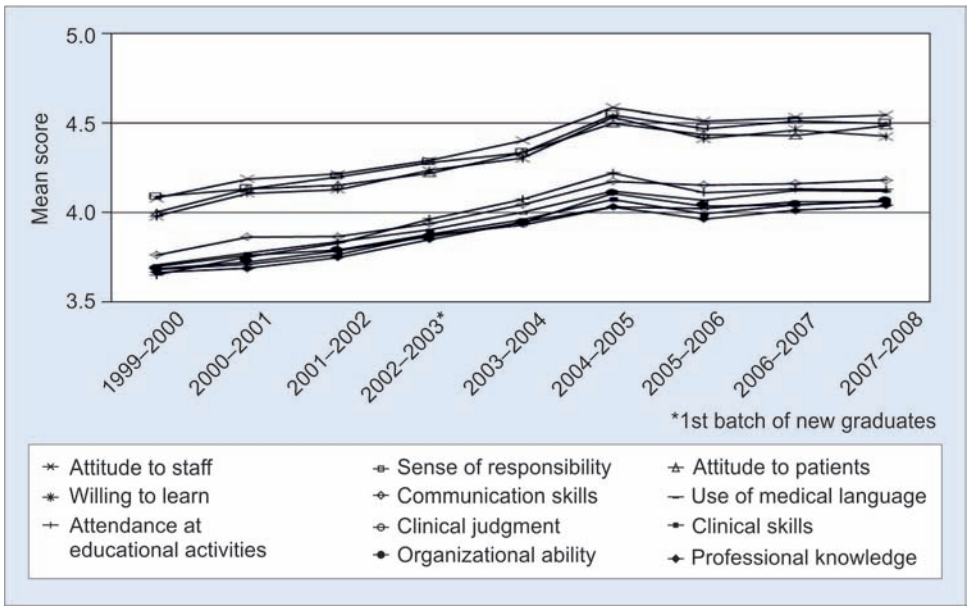
Internship Performance

Table 3 shows the results of t-test analyses. The mean scores of new curriculum were significantly higher than the old curriculum ($p < 0.01$), with effect sizes ranging from 0.46 to 0.57. Among the eleven domains, professional knowledge, clinical judgment, clinical skill and organizational ability were the areas of weakness within our graduates, no matter under the old or new curriculum. On the other hand, domains, such as attitude to staff, sense of responsibility, attitude to patients and willingness to learn were the areas of strength. The effect of new curriculum brought about seven percent

Table 3: Comparisons of internship performance between old and new curriculum

	Mean score			
Domains	*Old (99/00–01/02) n = 507*	*New (02/03–07/08) n = 969*	*Percent change*	*Effect size*
Attitude to staff	4.16	4.48*	7.69	0.52
Sense of responsibility	4.14	4.44*	7.25	0.46
Attitude to patients	4.10	4.40*	7.32	0.53
Willing to learn	4.07	4.39*	7.86	0.51
Communication skills	3.83	4.11*	7.31	0.47
Use of medical language	3.77	4.06*	7.69	0.53
Attendance at educational activities	3.75	4.10*	9.33	0.57
Clinical skills	3.75	4.00*	6.67	0.52
Organizational ability	3.74	4.01*	7.22	0.46
Clinical judgment	3.72	4.00*	7.53	0.52
Professional knowledge	3.71	3.97*	7.01	0.54

'*' represents that mean score of new curriculum was significantly higher than the old curriculum, analyzed by t-test ($p < 0.01$)



Graph 2: Internship performance from the year 1999–2000 to the year 2007–2008

improvement in the mean score in most of the domains. Ten percent improvement was obtained in the domain attendance at educational activities (Table 3).

A time-course for internship performance (mean scores of various domains) taken subsequently over 9 years has shown that mean scores tend to increase significantly over time from the first batch of new curriculum graduates (2002/2003) at all the domains ($p < 0.05$, by ANOVA). However, mean scores reached maximum in the year 2004/2005, and maintained a relatively stable level over the next 3 years (Graph 2).

DISCUSSION AND SUMMARY

It has been nearly 18 years, since the so-called new curriculum was introduced at the faculty of medicine, The University of Hong Kong. With further consolidation over the years, the curriculum has now become well established competency-based curriculum, incorporating online curriculum map. Although some studies in literature had questioned and expressed concern about the negative effect of PBL curriculum, which may result in weaker knowledge-base in students, especially of basic medical sciences,[4-7] the statistical analyses of the cumulative scores on knowledge-based assessment of old and new curriculum graduates, in this study, did not reveal any significant difference. The findings also reflected, that reduced number of didactic lectures to around 60% and selected practicals did not make students to 'learn less' in basic medical sciences. Also, there was a positive effect on knowledge application as witnessed during the professional activities of the interns.

In conclusion, initial pain and hard work of curriculum reform ultimately resulted into a joyous venture.

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CASE REPORT

Hungry Bone Syndrome due to Primary Parathyroid Adenoma with Multiple Bone Fractures

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ABSTRACT

Hungry bone syndrome (HBS) refers to the rapid, profound, and prolonged hypocalcemia associated with hypophosphatemia and hypomagnesemia which follows parathyroidectomy in patients with severe primary hyperparathyroidism (PHPT) and preoperative high bone turnover. It is a relatively uncommon, but serious adverse effect of parathyroidectomy. The severe hypocalcemia is believed to be due to increased influx of calcium into bone, due to the sudden removal of the effect of high circulating levels of PTH on osteoclastic resorption, leading to a decrease in the activation frequency of new remodeling sites and to a decrease in remodeling space, although there is no good documentation for this. Various risk factors have been suggested for the development of HBS, including older age, weight/volume of the resected parathyroid glands, radiological evidence of bone disease and vitamin D deficiency. The syndrome is reported in 25 to 90% of patients with radiological evidence of hyperparathyroid bone disease *vs* only 0 to 6% of patients without skeletal involvement. There is insufficient data-based evidence on the best means to treat, minimize or prevent this severe complication of parathyroidectomy. Treatment is aimed at replenishing the severe calcium deficit by using high doses of calcium supplemented by high doses of active metabolites of vitamin D. Preoperative treatment with bisphosphonates has been suggested to reduce postoperative hypocalcemia, but there are to date no prospective studies addressing this issue.

Keywords: Bisphosphonates, Hyperparathyroid bone disease, Osteoclastic resorption, Parathyroidectomy, Postoperative hypocalcemia.

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INTRODUCTION

The primary hyperparathyroidism is characterized by hypercalcemia due to increase of osteoclastic bone resorption. Parathyroid resection is the treatment of choice for patients of hyperparathyroidism with declining cortical bone density, nephrolithiasis and severe hypercalcemia.[1] One of the common complications of parathyroid surgery is the development of hypocalcemia. It is hypocalcemia varies, due to the possible surgical removal of all parathyroid tissue and long-term hypercalcemic suppression of nonadenomatous parathyroid glands.[1,2] Alternatively, hypocalcemia may be due to hungry bone syndrome (HBS), which is caused by massive calcium deposition in the bones after surgical treatment for PHPT.[1] We report a case of prolonged HBS in a 16-year-old female with a parathyroid adenoma.

CASE REPORT

A 16-year-old girl came to the Emergency department on 16th December 2013 with the complain of severe pain in lower limbs with shortening of right lower limb. There was no familial history of any form of hyperparathyroidism. Patient was admitted 2 years back with a 6-month history of intermittent abdominal pain and easy fatigability operated for acute pancreatitis. Two months after the surgery suffered femur neck fracture following a trivial fall. The fracture was treated with closed reduction with internal fixation at MGM Hospital, CBD Belapur, Navi Mumbai, India. On physical examination, there was a bilateral genu varus, bilateral tibia vara, ulnar deviation at both wrists, bilateral valgus elbow deformity and spine deformity with anterior neck swelling in the region of thyroid which was soft and smooth surfaced. Her height was 132 cm, weight 22 kg, and blood pressure 96/68 mm Hg. Biochemical investigations are shown in Table 1. Elevated serum calcium levels, decreased serum phosphate concentrations, together with increased PTH level confirmed the diagnosis of PHPT. Ultrasound demonstrated large parathyroid adenoma of size 3.5 × 1.2 cm on the left side. Computerized Tomography (CT) neck confirmed the ultrasound findings. It showed a 2.1 × 2.1 × 2.3 cm mass on left side of neck displacing left thyroid gland anteriorly. Roentgenogram of the long bones of upper and lower extremities and spine revealed demineralization with cystic lesions and multiple fractures (Fig. 1). She underwent surgery where on neck exploration, a well defined mass of parathyroid, 2 × 3 × 2.5 mm

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Table 1: Biochemical characteristics of the patient

	Parathyroid surgery		
	Before	*After*	*Reference range*
Serum total calcium (mg/dl)	12.62	6.4	8.8–10.6 mg/dl
Serum alkaline phosphatase (U/L)	2356	234	80–300 U/L
Serum PTH (pg/ml)	>1900	96	15–68.3 pg/ml
Blood urea nitrogen (mg/dl)	6.1	7.4	5–15 mg/dl
Serum vitamin D3 (ng/ml)	25.490	32.868	30–100 ng/ml
Spot urine Ca/Cr			

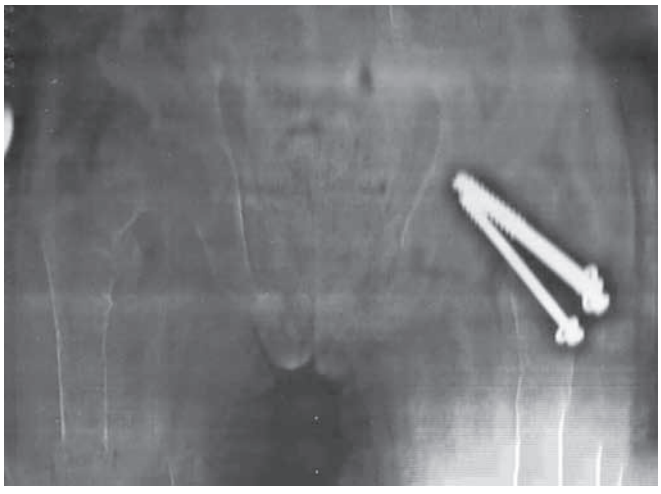

Fig. 1: Pretherapy X-ray pelvic both hips showing gross osteoporosis

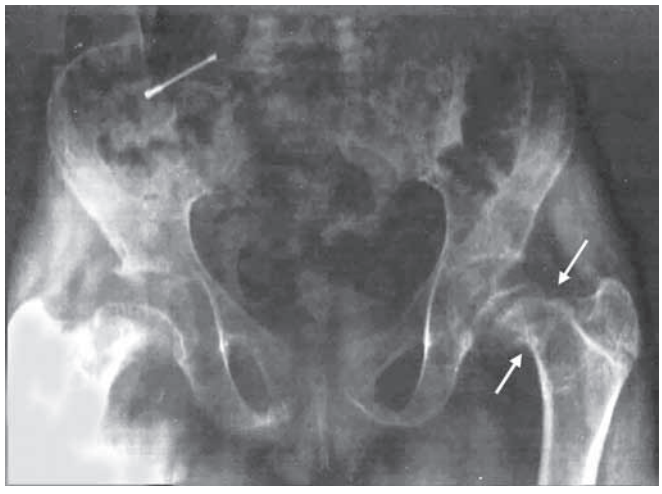

Fig. 2: Post-therapy X-ray pelvic both hips showing mineralization and improved bone density

in size, weighing 12 gm was excised. Light microscopic examination of the mass revealed a parathyroid adenoma. On the first postoperative day, the serum calcium level fell rapidly to 6.2 mg/dl.

Despite intravenous intermittent calcium supplementation, the serum calcium level remained less than 7.0 mg/dl and urinary calcium/creatinine ratio was consistently less than 0.05. Continuous intravenous and oral calcium supplementation was given for 20 weeks in form of injectable calcium gluconate in four divided doses of 200 mg per kg per day intravenously and calcium carbonate in two divided doses of 1 gm per day per orally respectively was given for 20 weeks and stopped. When serum phosphorus and alkaline phosphatase levels returned to normal limits 5 months after the operation, she had no symptoms and radiological evidence of mineralization of bone (Fig. 2).

DISCUSSION

Hungry bone syndrome is considered to be present if the serum calcium levels are below 8.5 mg/dl and the serum phosfate levels are normal or below 3 mg/dl on the third day after parathyroidectomy.[1] The predominant feature of the present case was the marked and a longstanding postoperative HBS. It can be explained by the long period of hypercalcemia secondary to parathyroid adenoma that resulted in atrophy of the healthy parathyroid glands. One previous report demonstrated HBS persisting for 27 weeks postsurgery.[3] Smith et al recommended that preoperative treatment with calcitriol for 5 to 10 days may prevent HBS in the postparathyroidectomy state.[4] In primary hyperparathyroidism, 25-hydroxy vitamin D concentration tends to below normal, while 1,25-dihydroxy vitamin D tends to be high normal.[5] Bisphosphonates have a negative effect on bone remodeling and some authors recommended their use to prevent HBS in patients with PHPT.[6,7] Hungry bone syndrome secondary to PHPT is transient. In this period, calcium supplementation is preferred over bisphosphonate treatment in children.

Brasier et al[1] followed 198 adult patients after surgery for PHPT and studied the risk factors for the development of HBS.[1] They reported a positive correlation with ageing, larger adenoma size, increased serum alkaline phosphatase levels, and elevated blood urea nitrogen levels. There has been no report on predictive risk factors for HBS in children with PHPT. Because bone metabolism is more active in children than in adult patients, HBS is more severe and frequent in young patients with PHPT.[5,8] Although, most patients with primary hyperparathyroidism demonstrate preserved vertebral bone marrow density (BMD), which mainly reflects cancellous bone, but this patient showed a significant loss of vertebral bone

density along with appendicular skeleton. There are a few reports on severely affected lumbar spine bone densitometry in adults with PHPT, but no report in children with PHPT is available.[9] This finding may indicate that severely affected lumbar spine BMD at the diagnosis of PHPT may be used as one of the additional preoperative predictors of HBS in children. The presentation of PHPT in children is different from PHPT in adults, in whom the disease is usually less severe.[10]

CONCLUSION

It has been found that there was a reversible cancellous BMD loss in our patient with PHPT. Overt bone disease, raised alkaline phosphatase, decreased cancellous BMD and a large parathyroid adenoma may be used as preoperative predictive risk factors of HBS in pediatric patients with PHPT.

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CASE REPORT

Non-mycosis Peripheral T-Cell non-Hodgkin Lymphoma involving the Skin

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ABSTRACT

A 64 years old male presented with reddish lesions all over the body of 1 month duration, high grade fever with evening rise of temperature and chills. No lymphadenopathy or hepatosplenomegaly were noted. Multiple infiltrated erythematous and hyperpigmented patches and plaques were present on the face, trunk and extremities along with few oral erosions. Histopathology from skin showed features of mycosis fungoides (MF). A further workup with Immunohistochemistry was suggestive of peripheral T-cell lymphoma, not otherwise specified diagnosis (PTCL-NOS). We report a case of PTCL-NOS in a man mimicking MF clinically and histopathologically.

Keywords: Peripheral T-cell lymphoma (PTCL), Peripheral T-cell lymphoma-not otherwise specified (PTCL-NOS).

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INTRODUCTION

Peripheral T-cell lymphomas (PTCL) comprise a rare and heterogeneous subset of T-cell non-Hodgkin lymphomas (NHL) which comprise of a diverse group of disorders that, for the most part, carry a poor prognosis. They arise from lymphocytes at the post-thymic stage of maturation, at nodal or extranodal sites and display T-cell/NK-cell immunophenotype.[1] It is distinct from the more common cutaneous TCL.[1] Any mature T-cell NHL is considered a PTCL, with the exception of lymphoblastic lymphoma. We report a case of PTCL, not otherwise specified (PTCL-NOS) in a man mimicking mycosis fungoides (MF) clinically and histopathologically.

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CASE REPORT

A 64 years old male presented with reddish lesions all over the body of 1 month duration. The lesions began appearing on the abdomen and spread all over the body in a duration of 15 days. Patient gave history of high grade fever, along with evening rise of temperature and chills. In addition, he complained of oral lesions and nasal stuffiness. There was history of joint pain 1 week following development of skin lesions. Patient was a known case of hypertension on treatment with calcium channel blockers.

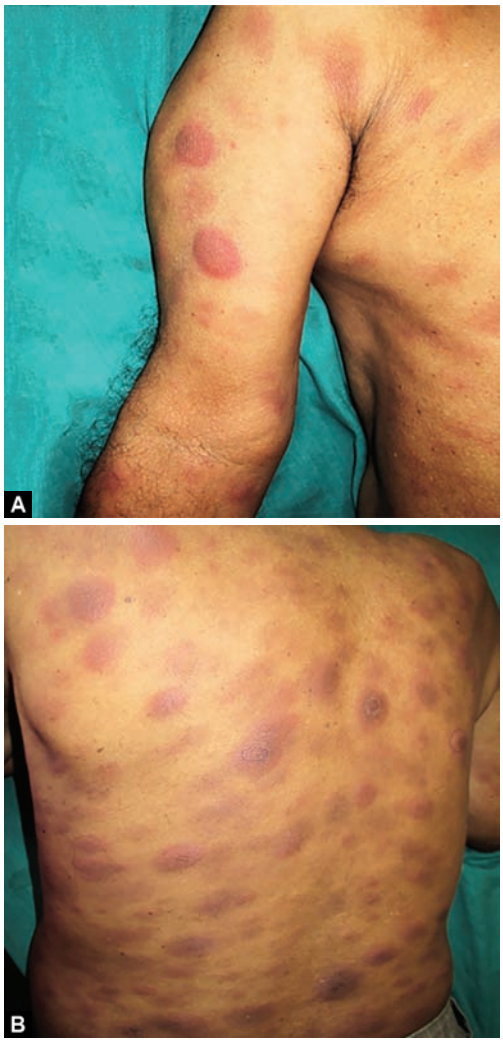
Clinical examination revealed fever (101°F) along with tachycardia. No lymphadenopathy or hepatosplenomegaly were noted. Lesions were generalized and distributed bilaterally symmetrical on the face, trunk and extremities. Multiple infiltrated erythematous and hyperpigmented patches and plaques were present (Figs 1A and B). Oral cavity showed few erosions on the palate.

Patient's hemogram showed neutrophilia, but the total count was within normal limits. Serum urea, creatinine and liver enzymes were slightly elevated. Erythrocyte sedimentation rate (ESR) was also elevated (60 mm). Histopathology from skin was suggestive of MF (Figs 2 and 3). Immunohistochemistry (IHC) showed predominant periadnexal infiltrate of atypical lymphoid cells marking positive for CD3, CD5, CD45, CD2, CD4, CD7 and TCRβF1 and negative for CD8, CD10, CD20, CD30 and CD23 negative. Mib-1 was 80%. Non-mycosis peripheral T-cell non-Hodgkin lymphoma involving the skin was described and based on the IHC markers, a final diagnosis of PTCL-NOS was made.

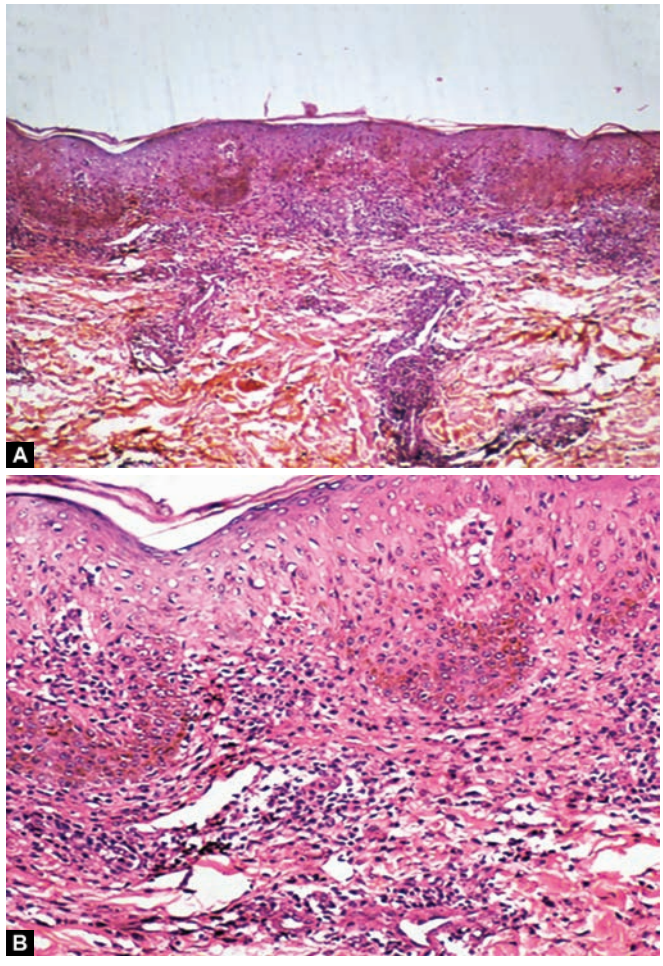
Prior to IHC report, patient was treated symptomatically. He did not develop fresh lesions, but continued to have evening rise of temperature. He was discharged against medical advice and succumbed to the disease within one and a half months of admission.

DISCUSSION

Peripheral T-cell lymphoma are uncommon, accounting for only 10 to 20% of all NHLs[2] with an incidence of less than one case per 100,000 in the US.[3] A large clinicopathologic study[4] of PTCL and natural killer/T-cell lymphoma (NKTCL) reported that the most common PTCL subtypes are PTCL-NOS (25.9%), angioimmunoblastic T-cell lymphoma (AITL) being the second



Figs 1A and B: Multiple infiltrated erythematous and hyperpigmented patches and plaques over the upper limbs and trunk



Figs 2A and B: The epidermis shows acanthosis and infiltration with inflammatory infiltrate which is also seen at the dermoepidermal junction and around the adnexa (H and E, 4×); b) Lymphoid tumor cells in singles, clusters and sheets in the dermis, more around the adnexa (H and E, 10×)

most common (18.5%). Natural killer/T-cell lymphoma represented 10.4% and adult T-cell leukemia/lymphoma (ATLL) 9.6% of the cases. However, studies have shown that PTCL and NKTCL which are common in many other Asian countries are less prevalent in India.[5,6]

World Health Organization (2008) revised the classification of PTCL (Table 1) through a combination of morphologic, immunophenotypic, genetic, molecular, and clinical features, thus defining many additional subtypes.[7] Peripheral T-cell lymphoma-not otherwise specified include all cases not readily classifiable into other specific T-cell entities of the WHO classification and primary presentation in the skin as in this case is uncommon. Peripheral T-cell lymphoma-not otherwise specified typically occurs in adults at the median age of 55 to 60 years, and a higher prevalence is seen in males[8,9] which was consistent with our case. Complete clinical examination and an accurate clinical history are critical to particularly rule out (transformed) MF or one of the three subtypes of primary cutaneous PTCL that have been recognized in recent classifications, i.e. primary cutaneous γδ T-cell lymphoma (PCGD-TCL), primary cutaneous $CD8^+$ aggressive epidermotropic cytotoxic T-cell lymphoma (PCAEC-TCL), and primary cutaneous $CD4^+$

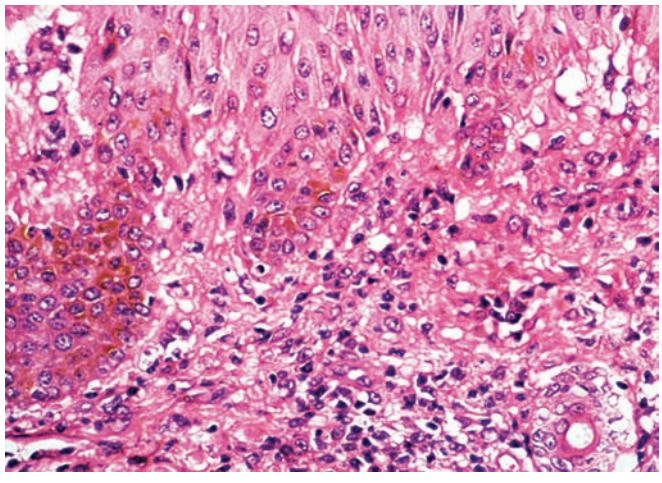


Fig. 3: Tumor cells were small round to medium sized lymphoid cells, having hyperchromatic and cerebriform nuclear contours, few of them showing prominent nucleoli (H and E, 40×)

Table 1: World Health Organization classification of peripheral T-cell lymphoma (2008)[7]

Type	*Subtype*
T-cell prolymphocytic leukemia	—
T-cell large granular lymphocytic leukemia	—
Chronic lymphoproliferative disorder of NK cells*	—
Aggressive NK-cell leukemia	—
Systemic EBV-positive T-cell lymphoproliferative disease of childhood	—
Hydroa vacciniforme-like lymphoma	—
Adult T-cell leukemia/lymphoma	—
Extranodal NK/T-cell lymphoma, nasal type	—
Enteropathy-associated T-cell lymphoma	—
Hepatosplenic T-cell lymphoma	—
Subcutaneous panniculitis-like T-cell lymphoma	—
Mycosis fungoides	—
Sézary syndrome	—
Primary cutaneous $CD30^+$ T-cell lymphoproliferative disorders	Lymphomatoid papulosis
	Primary cutaneous anaplastic large cell lymphoma
Primary cutaneous γδ T-cell lymphoma	—
Primary cutaneous $CD8^+$ aggressive epidermotropic cytotoxic T-cell lymphoma*	—
Primary cutaneous $CD4^+$ small/medium T-cell lymphoma*	—
Peripheral T-cell lymphoma, NOS	—
Angioimmunoblastic T-cell lymphoma	—
Anaplastic large cell lymphoma	ALK-positive
	ALK-negative*

NOS: Not otherwise specified; ALK: anaplastic lymphoma kinase; and NK: natural killer; *These histologic types are provisional entities for which the WHO working group felt there was insufficient evidence to recognize as distinct diseases at this time

small/medium pleomorphic T-cell lymphoma (PCSM-TCL).[10] Primary cutaneous γδ T-cell lymphoma and PCAEC-TCL were ruled out on the basis of IHC markers (TCR-γδ positive and CD8 negative respectively), whereas PCSM-TCL on the basis of clinical features (presents with a solitary plaque or tumor that is generally localized to the head or neck).

Variable clinicopathological characteristics necessitate the use of IHC panels for the diagnosis of PTCLs. Moreover, even the IHC markers show considerable degree of overlap. Hsi et al[11] recommended a two tier diagnostic approach for IHC panels: Panel 1 consists of IHC (CD3, CD5, CD10, CD20, CD21, CD30, CD45, PAX5), and panel 2 consists of IHC (CD2, CD4, CD7, CD8, CD23, PD-1, CD56, EBER, ALK1, TIA1, TCRγ, TCRβF1) markers.

As a group, PTCL-NOS are aggressive neoplasms and often present with advanced stage.[8,12] The overall survival rates of PTCL-NOS presenting in the skin are poor and independent of the presence or absence of extracutaneous disease at the time of diagnosis, cell size, or expression of $CD4^+$ or $CD8^+$ phenotype.[10]

In conclusion, we highlight the importance of this rare, aggressive entity with poor prognosis as a differential for the more commoner and indolent MF.

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CASE REPORT

Successful Pregnancy and Delivery with Good Maternal and Fetal Outcome in a Kidney Transplant Recipient

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ABSTRACT

Conception and successful completion of pregnancy is rare in women with end-stage kidney disease. Given the rising burden of chronic kidney disease, it is quite common to see more women in their childbearing ages being diagnosed with the condition. As the kidney disease progresses, fertility chances reduce and pregnancy becomes a rarity. In addition to dealing with dialysis and its consequences, the women with end-stage kidney disease also face the trauma of infertility and inability to start their families. At such times, pregnancy and delivery following successful kidney transplantation with return of normal kidney function, offers a ray of hope to women of childbearing ages. We report the case of a young woman with end-stage renal/kidney disease (ESRD) on hemodialysis for 2 years, who underwent cadaveric kidney transplantation with subsequent excellent allograft function. Two years post-transplantation, she went ahead with a successful pregnancy and delivery of a normal birth weight baby, and preserved renal allograft function.

Keywords: Kidney transplant recipient, Maternal outcomes, Pregnancy.

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INTRODUCTION

Case History

A 25-year-old married woman was seen in gynecology clinic for evaluation of infertility in 2010. She denied any symptoms other than an occasional headache, anorexia and weakness. Preliminary blood tests showed markedly abnormal renal parameters with blood urea nitrogen (BUN) of 60 and serum creatinine of 6.5. She was referred to Nephrology. She had no prior history of hypertension, diabetes, hepatitis or nephrolithiasis. She denied any specific urinary symptoms. Family history was non-contributory and she was not taking non-steroidal anti-inflammatory drugs or any herbal supplements. Upon physical examination, significant findings were pallor and hypertension. A sonography of the kidneys revealed bilateral, small and shrunken kidneys with increased cortical echogenicity without any hydronephrosis or calculi. Urine analysis showed proteinuria without any casts or crystals. The 24 hours urine sample showed 1.2 gm of proteinuria. In addition to the raised BUN and serum creatinine, other complications of renal dysfunction, like anemia, metabolic acidosis and elevated potassium were also noted. A diagnosis of end-stage renal disease (ESRD) was made and further treatment options were discussed. She was started on antihypertensive medications, sodium bicarbonate tablets, and phosphorus binder tablets. Iron and erythropoietin injections were added. Given the absence of any reversible causes of renal dysfunction and the small sized shrunken kidneys seen on sonography, renal replacement therapy options were considered. Kidney transplant options were discussed with the patient and her family. Her blood group was O^+. Given no potential living donors in the family, she was placed on a cadaver transplant wait list with zonal transplant co-ordination centre and MGM New Bombay Hospital, Vashi. A left arm arteriovenous fistula was placed, and hemodialysis treatments started twice a week for azotemia and uremic symptoms.

After being on hemodialysis for nearly a year and a half, she received a call from MGM New Bombay Hospital, Vashi, regarding the availability of a blood group-matched cadaver donor. She underwent a cadaveric kidney transplant surgery in June 2012. The donor was a 19-year-old, blood group O^+ female, declared brainstem dead following a road traffic injury. The transplanted kidney functioned well with good urine output and steady decline in serum creatinine values. Her immunosuppressive medicines included tacrolimus, mycophenolate mofetil and prednisolone. Post-discharge, she continued with regular follow-up and the renal allograft function was excellent with a baseline serum creatinine of 0.7 to 0.8. She had mild hypertension, which was treated with metoprolol.

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She expressed her desire to start a family, but was advised to wait for at least a year post-transplant and continue with contraception in the interim. After about 2 years, given excellent allograft function with no episodes of rejection or infections, she was permitted to try for pregnancy. For planning a pregnancy, her immunosuppressive medications were changed; mycophenolate mofetil was discontinued and azathioprine was started. Prednisolone and tacrolimus were continued. Metoprolol was switched to labetalol. The high-risk nature of the pregnancy was explained to the family alongwith the need to closely monitor the patient during the pregnancy; especially to monitor kidney graft function, infection, rejection or development of pre-eclampsia.

She conceived in March, 2014. During pregnancy, she was closely followed-up both by a obstetrician and a nephrologist. Blood pressure and renal function were monitored regularly. Pregnancy course was uneventful with no infections, graft rejection or pre-eclampsia. Transplant kidney function remained stable. In the third trimester, she developed gestational diabetes that responded well to diet control and reduction in tacrolimus dose. Blood pressures were elevated, requiring increases in the labetolol doses. In the 35th week of pregnancy, she was taken up for cesarean section, given worsening blood pressure (BP) though no proteinuria. Stress dose steroids were given intraoperatively. She delivered a healthy 2.5 kg weight baby in December, 2014.

Post-pregnancy, her renal allograft function remains stable. Breast feeding has been deferred given need to continue immunosuppressive medicines that get excreted in the milk. Her kidney function remains stable with a serum creatinine of 0.7. She has been switched back to mycophenolate mofetil from azathioprine. Both mother and baby are doing well.

DISCUSSION

Kidney disease is associated with a risk of both maternal and fetal adverse outcomes. Pregnancy in a woman with kidney disease can increase the risks for gestational hypertension, pre-eclampsia and eclampsia. Pregnancy confers a serious risk of worsening of kidney function during pregnancy in women with pre-existing moderate to severe chronic kidney disease (defined as serum creatinine > 1.3 mg/dl and > 1.9 mg/dl respectively).[1] Fetal risks include intrauterine growth retardation, preterm birth and still birth.

Pregnancy in end-stage kidney disease (ESRD) population is uncommon.[2] Infertility rates are high in them, and according to a study; the reported frequency of conception in women on dialysis is around 0.3 to 1.5% per year.[3] Rate of fetal deaths during pregnancies in dialysis patients is significantly high although more recent data suggest improved outcomes with higher rates of live births (40–86% of all pregnancies).[4,5] The improved pregnancy outcomes in women with ESRD on dialysis is thought to be due to an aggressive management of uremia with intensified dialysis keeping target blood urea levels lower than 50 mg/dl (recommended measures include dialysis more than 36 hours per week, long nocturnal dialysis and more frequent dialysis 4 to 6 times/week, etc). Additional suggested measures include correction of metabolic acidosis and hypocalcemia, targeting hemoglobin between 10 and 11 g/dl with erythropoietin injections, avoidance of hypotension during dialysis and careful monitoring of nutrition and adequate protein intake by the mother. These measures are intended to provide the best possible environment to the developing fetus to ameliorate polyhydramnios, improve maternal blood pressure and increase the gestational age and fetal birth weight.[5-7] However, despite these improved fetal outcomes, pregnancy in women on dialysis poses a significant risk of severe hypertension, pre-eclampsia and premature delivery.[8] Under such circumstances, if a kidney transplant option is feasible, it would be the ideal situation for women of childbearing age with ESRD, to plan for pregnancy after successful kidney transplantation.

Post-transplantation, fertility is restored. However, the rate of successful continuation of pregnancy to term is still quite low as compared to the general population. According to a study, 55% of pregnancies in transplant recipients resulted in a live birth, as compared to nearly 70% in the general population.[9] In addition, the frequency of pre-eclampsia, intrauterine growth retardation and premature delivery are increased in transplant recipients.[9-13]

Traditionally, women were usually advised to wait at least two years after transplantation so as to avoid complications arising from immunotherapy and rejection.[14] However, longer wait times for a kidney transplant and increasing age of women pose reduced time for successful pregnancy post-transplantation for such women of childbearing age. According to American society of transplantation, consensus opinion, as long as graft function is optimal (defined as serum creatinine < 1.5 mg/dl, with < 500 mg/24 hours protein excretion), no use of teratogenic medications, and immunosuppressive medicines at stable levels, the patient can safely proceed with the pregnancy.[11,15] As in the case of chronic kidney disease, pregnancy has little or no effect on the kidney allograft function, provided that the allograft is functioning well at baseline. Hence, it is recommended that prior to contemplating pregnancy in a transplant recipient, the serum creatinine level should be stable and < 1.5 mg/dl, and urinary protein excretion < 500 mg/day.[11]

Specific maternal concerns in pregnant renal transplant women are—onset of hypertension, worsening of pre-existing hypertension, development of proteinuria and pre-eclampsia, renal allograft rejections, superimposed infections (especially urinary tract infections), and development of gestational diabetes.

Prevalence of hypertension is high among pregnant renal transplant women, up to 73% according to registry report.[16] In addition, renal transplant patients with hypertension are at higher risk for development of superimposed pre-eclampsia with an incidence of 15 to 25% as compared to 5% of normotensive pregnancies.[17] Hence, close monitoring of blood pressure and use of approved antihypertensive medications during pregnancy for BP control is mandatory in the management of pregnant renal transplant recipient.

Renal allograft rejection is another risk during pregnancy, especially because changes in blood volume can alter serum levels of immunosuppressive medications. Thus, immunosuppressive drug levels are checked frequently during pregnancy for dose titration.[11,18-20] A transplant renal biopsy can be undertaken during pregnancy, if there are concerns of rejection and appropriate treatment can be started.

Pregnant renal transplant women are at higher risk for developing gestational diabetes, hence, screening glucose tolerance test is recommended each trimester.[21] Among infections, urinary tract infections are seen more frequently in pregnant renal transplant recipients.[22] Hence, screening and treatment of asymptomatic bacteriuria is also recommended.

Specific concerns to the fetus in pregnancies post-renal transplantation include—preterm delivery, low birth weight and intrauterine growth retardation.[18,21] Thus, serial sonographic surveillance of the fetus is carried out during such pregnancies.[21] In addition, effect of immunosuppressive medications on the developing fetus is a major concern. Among the immunosuppressive medicines, mycophenolate mofetil in particular has been associated with structural malformations in the fetus and its use is contraindicated in pregnancy.[23] Mycophenolate should be discontinued at least 6 weeks prior to planning pregnancy. Azathioprine is safer and can be used as a substitute to mycophenolate mofetil during pregnancy. Calcineurin-inhibitors like tacrolimus and cyclosporine are used during pregnancy, although their levels should be closely monitored and doses adjusted to avoid toxicity as well as maintain adequate immunosuppression during pregnancy.[11,20]

Breastfeeding is generally deferred in kidney transplant recipients due to the immunosuppressive medications being secreted in breast milk and risk of exposure to the baby. However, due to lack of data and controlled studies on the pharmacokinetics and levels of immunosuppressive medications in breast milk, there is no expert consensus that absolutely contraindicates breast feeding in women on immunosuppressive medications.[11,15]

CONCLUSION

A successful kidney transplant offers an opportunity to young women with advanced kidney disease to contemplate pregnancy and delivery. Although lower than the general population, the successful maternal and fetal outcomes post-pregnancy in a kidney transplant recipient certainly gives them a ray of hope. Such pregnancies should be considered high-risk and monitored closely both by the nephrologist and obstetrician, and the patient should be counseled appropriately.

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