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From Editor's Desk

Generalized obesity, in which there is increase in total body fat, is a well-recognized major risk factor for type 2 diabetes, coronary artery disease, hypertension, and other related metabolic disorders. Global incidence of obesity has increased to near epidemic proportions, which correlates well with increasing incidence of the disorders mentioned above. During last 30 years, it is becoming increasingly apparent that visceral obesity, in which there is excess fat deposition in the abdominal cavity, bears greater correlation with the prevalence of above-mentioned comorbidities than generalized obesity per se. In fact, waist circumference and/or waist/hip circumference ratio, when added to body mass index, has been found to be a more sensitive predictor of developing cardiometabolic disorders like, insulin resistance, type 2 diabetes, cerebrovascular stroke, coronary artery disease, and hypertension. Numerous studies have been carried out and many more are in progress as to why it is so. It seems likely that visceral obesity may not be the direct cause of these disorders, but more probably it may be a marker of abnormal pattern of fat deposition in which fat tends to accumulate in and around viscera and skeletal muscles rather than subcutaneous tissues. Several approaches to deal with this malfunction in pattern of fat deposition are under investigation. Lifestyle modification in the form of nutritional interventions and increased physical exercise is of utmost importance. In addition, several pharmacotherapeutic approaches are being studied, like use of serotonin 2C receptor antagonist, thiazolidinediones, growth hormone, androgens, and cortisol inhibitors.

In this issue of MGMJMS, two interesting articles pertaining to obesity have been published. First article describes the use of indigenously developed body component analyzer developed by Bhabha Atomic Research Center, Mumbai in measuring visceral fat by using bioimpedance technique. Second article reports about plant-derived pancreatic lipase inhibitors which can be used safely as anti-obesity drugs. In addition, there is a usual mix of original articles, review articles, case reports, and short communication.

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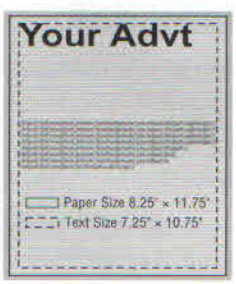

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ORIGINAL ARTICLE

Effects of Subacute Exposure to Gold Nanoparticles on Germ Cells of Zebrafish (*Danio rerio*): An *in vivo* Study

[1]Navami Dayal, [2]Mansee Thakur, [3]Poonam Patil, [4]Niharika Swain, [5]Dattatraya Shankar Joshi

ABSTRACT

Although *in vivo* studies have been modeled using higher mammal systems, the lower vertebrate zebrafish *(Danio rerio)* has gained tremendous attention as a model system. Gold nanoparticles (GNPs) attract the interest of scientists due to their promising potential applications in medicine and targeted drug delivery. The purpose to use GNPs *in vivo* is that gold in bulk form is nontoxic and apply the positive potentials of nanoparticles. Bulk gold is century-long accepted as a safe-to-use metal. Gold in its nanoform has distinct chemical and physical properties and the large amount of surface atoms make GNPs reactive. Moreover, GNPs can potentially access many cellular or subcellular structures, which are unreachable by the larger compound and may induce toxic effects. This paper addresses effects of spherical GNPs of average size 15 nm on reproductive organs after subacute exposure in adult male and female zebrafish. Gold nanoparticles were chemically synthesized and characterized by transmission electron microscope.

The primary objective of this study was to determine if exposure to GNPs altered cellular morphology of gonads. The adult fish of both sexes were administered orally with these GNPs at a dose of 20 µg/gm. At the end of the study, quantification of gold content was estimated using two different tools: inductive coupled plasmon-atomic emission spectroscopy (ICP-AES) and inductive coupled plasmon-mass spectroscopy (ICP-MS). No gold metal accumulation was detected in treated group of male and female zebrafish at subacute exposures on estimation through ICP-AES. On analysis using ICP-MS, 0.44 ± 0.18 µg/gm organ weight was detected in ovaries and 4.6 ± 3.20 µg/gm organ weight was detected in testes of treated groups. However, the pattern of accumulation was found to be nonsignificant when compared with the control group at a p-value >0.05. Histopathological analysis of reproductive organs showed no significant changes in cellular morphology of testes and ovaries.

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INTRODUCTION

Gold nanoparticles (GNPs) have traditionally been considered inert and biocompatible. Its high surface area and volume ratio has resulted in its wide applications in biomedical research.[1] Gold nanoparticles of various size and shape have attracted considerable interest for medical applications, e.g., as carrier for drugs, such as paclitaxel, tumor-detector, photothermal agent or radiotherapy dose enhancer.[2,3] Despite their huge potential benefits in the biomedical applications, very little is known about the short and long-term health effects in organisms.[4,5] Bulk gold was used *in vivo* in the 1950s and was considered nontoxic, but functionalized gold particles showed obvious cytotoxicity.[6,7] An increasing number of scientific reports have appeared in the last decade that highlight the issue of understanding the interactions between different types of nanoparticles and cells as functions of size, shape, and surface chemistry of the nanomaterial.[8] The unexpected accumulation of nanoparticles in organs has a potential risk to induce organ dysfunction and diseases.[9,10] Unfortunately, no simple conclusions have emerged from the available studies due to the variability of parameters, such as the physical and chemical properties of the particle, cell type, dosing parameters, and the biochemical assays used. Moreover, the majority of the scientific reports that investigate the cellular impact of nanomaterials are *in vitro*, with far less effort to understand the real situation *in vivo*.[11] Some of the crucial issues that need to be addressed for toxicity assessments of nanomaterials are effect of shape and size, dosimetry, route of delivery and tracking, development, and validation of test models, *in vitro vs in vivo* extrapolation, etc.

Currently, small animal models are the "gold standards" for nanomaterial toxicity testing. Recently,

zebrafish is becoming a useful vertebrate model system for increasing number of studies on many of the human diseases, drug discovery, drug delivery systems, toxicity assessments, etc.[12,13] Apart from reports on general systemic toxicity of GNPs, there is a paucity of data available on reproductive toxicity associated with GNPs and lack of safety and regulatory guidelines concerning application of GNPs in consumer products. This highlights the need to consider not only the usefulness of NP but also the potentially unpredictable and adverse consequences of human exposure thereto. This applies especially to their potential reproductive toxicology (nanoreprotoxicity), because any shortcomings in this regard would be reflected into the next generation. Moreover, the 1 to 100 nm scale is of interest for biological interfaces; e.g., objects less than 12 nm in diameter may cross the blood-brain barrier[14-16] and objects up to 45 nm can be endocytosed by cells while those more than 70 nm tend to remain on the surface of the cells.[17] Therefore, the purpose of this research is to utilize zebrafish as an *in vivo* vertebrate model in a subacute (14 days) study to assess bioavailability and toxic effects of GNPs with an average size of 15 nm since it is known to have more potential to cross the physiological barriers.

MATERIALS AND METHODS

Gold Nanoparticles Synthesis and Characterization

To produce small nanoparticles, the procedure of the Turkevich et al was used.[18,19] Briefly, 10 mL of 1 mM tetrachloroaurate is heated to near boiling (96°C) followed by addition of 1 mL of 41 mM trisodium citrate. Solution was stirred vigorously on a magnetic stirrer with heating mantle till about 8 to 10 minutes, and then allowed to cool at room temperature.

The formation of GNPs was monitored using double beam UV/visible spectrophotometer (Thermo Scientific, Evolution 201 series). The size and shape of the nanoparticles were confirmed using transmission electron microscopy (TEM) (Philip, Model No. CM200, operating voltages: 20 to 200 kV resolution 24 Å). This solution was stored at 4°C for further use. Stability of the suspension was monitored every week using UV-visible spectrophotometer and was found to be stable for 2 months.

Dose Determination: GNPs to be Administered to Adult Zebrafish

Prior to initiating studies on adult zebrafish, preliminary experiment was performed on zebrafish embryos to determine LC_{50} value for GNP's. For this purpose, five different test concentrations were selected with distilled water as a control. Three replicates of 20 zebrafish embryos were exposed per concentration at 4–6 hours post fertilization (HPF) as per the organization for economic cooperation and development (OECD) guidelines[20] and monitored for their viability at every 24 hours till 96 HPF.

Experimental Design

Animal experiments were performed in the zebrafish facility at the Central Research Laboratory, fulfilling the criteria of good laboratory practices. Experiments were designed according to OECD guidelines for fish (Test no. 204; 1984).[21] Indigenous wild type male and female adult zebrafish strains (3–4 months old) were used for this study. Fish was stocked in static systems with continuous supply of aeration under 14:10 hours light and dark cycle. They were fed twice a day by local fish feed and once with GNPs at an interval of 4 hours daily. During this period, the water temperature was maintained at 28 ± 1°C and no fish died throughout the test period. Studies were divided into two groups: Control group (males and females) and treated group (males and females).

Route of Administration of GNPs to Adult Zebrafish

For treated group, GNPs were administered orally according to the protocol explained previously[22] at a repeated dosing for duration of 14 days. At the same time, control groups were administered with equal volume of distilled water. Experiments for each group (control and treated) was conducted in triplicates with seven healthy zebrafish per sex.

Histological Examination

Histological examination was performed following 14 days of subacute exposure. For this purpose, the fish was anesthetized in ice water and dissected to obtain testes and ovaries. The organs were fixed in 10% formalin for 24 hours at room temperature. Fixed tissue was dehydrated and embedded in the paraffin wax. Serial cross sections of 5 µm were cut by microtome (Leica RM255) and stained with hematoxylin and eosin. The samples were examined under the light microscope (Olympus Magnus, Model no. 11F589). Staging of germ cells was observed as described by Menke et al 2003.[23]

Pattern of Bioaccumulation in Gonad

At the end of the test period, fish tissues (testes and ovaries) were sampled for estimation of gold content in respective organs. Prior to digestion for gold content

measurements, the samples were thoroughly rinsed using distilled water and dried for 48 hours at 55°C. After cooling, they were weighed followed by digestion of tissues in 3 mL HNO_3 (15.3 M) by heating at 90°C (180 min) on a sand bath.[24] After complete digestion, samples were then evaporated to incipient dryness (100°C). The digestion process was completed by the addition of 2 mL of H_2O_2 (1M) and evaporation to incipient dryness (60 min, 100°C). Prior to measurements by inductive coupled plasmon-atomic emission spectroscopy (ICP-AES) (ARCOS from M/s Spectro, Germany) and inductive coupled plasmon-mass spectroscopy (ICP-MS) (Thermo Fischer Scientific, Germany), acidified ultrapure water (2% v/v, HNO_3, 15.3 M) was added. Gold content in respective samples was measured to determine the accumulation pattern of the GNPs in testes and ovaries.

RESULTS

Synthesis and Characterization of Gold Nanoparticles

A simple one-step synthesis method for the preparation of uniform and stable GNPs was employed (Fig. 1A). The UV/visible spectrum of synthesized GNPs showed maximum absorbance at 520 nm (Fig. 1B). The TEM image and size distribution plot of GNPs indicated spherical shaped particles with an average diameter of 15 ± 5 nm (Figs 1C and D).

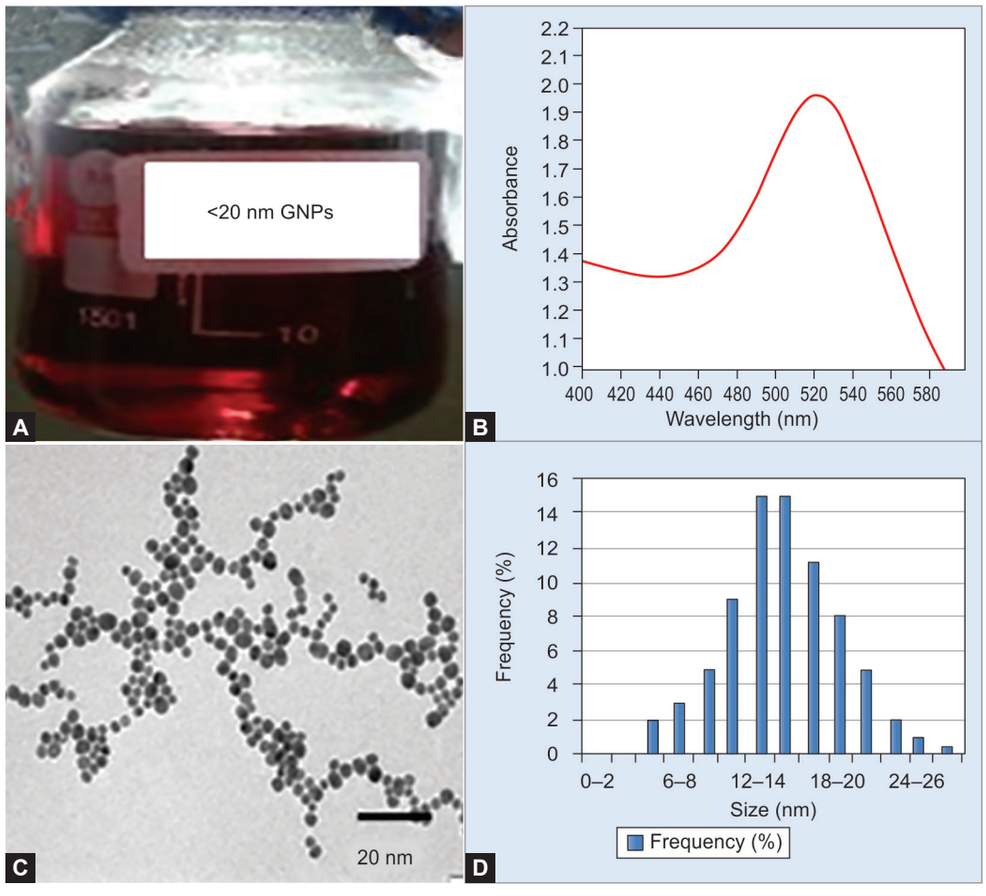
Dose Determination: GNPs to be Administered to Adult Zebrafish

Viability percentage of the embryos was determined at the end of 96 HPF to estimate the LC_{50} value for spherical GNP's with average diameter of 15 nm. LC_{50} for 15 ± 5 nm GNPs was obtained at 10 µg/mL (Fig. 2).

Thus, a concentration of 10 µg/mL was used for further studies on adult zebrafish. The average weight obtained for male and female zebrafish used in the present study was 0.5 gm, thus, the dose calculated is 10 µg/0.5 gm body weight of the fish, i.e., 20 µg/gm body weight of fish.

Gold Content Estimation in Gonads of Zebrafish

In order to validate the success of the protocol of oral administration whether GNPs have reached its target organ, i.e., gonads, the reproductive organs were dissected posttreatment followed by acid digestion and analyzed for accumulation of gold content using ICP-AES and ICP-MS. No gold metal accumulation was detected



Figs 1A to D: Synthesis and characterization of GNPs: (A) Chemically synthesized GNPs, (B) UV-visible spectrum with absorption maxima at 520 nm, (C) TEM image at a scale bar of 20 nm indicating GNPs with spherical shape, and (D) size distribution plot

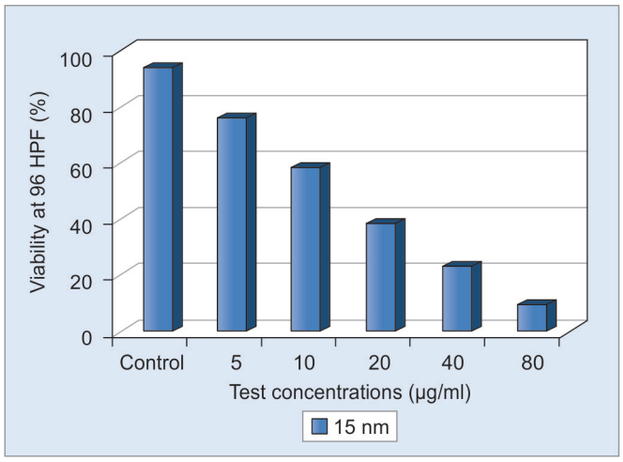


Fig. 2: Histogram showing percentage viability at 96 HPF for embryos exposed to spherical GNPs of average diameter of 15 nm

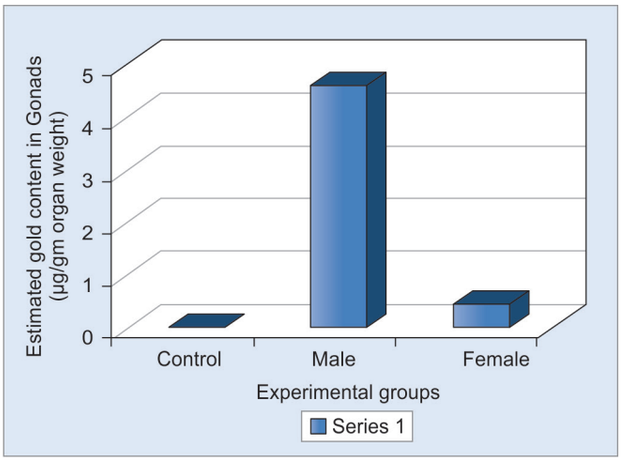


Fig. 3: Histogram plot for estimation of gold content (µg/gm organ weight) in Gonads using ICP-MS

Table 1: Estimation of gold content (µg/gm organ weight) in Gonads using ICP-AES and ICP-MS

Test	*Control*	*Male*	*Female*
ICP-AES	< 0.01	< 0.01	< 0.01
ICP-MS	< 0.01	4.6 ± 3.20	0.44 ± 0.1

Note: < 0.01 means not detected

in treated group of male and female zebrafish at subacute exposures on estimation through ICP-AES (Table 1). On analysis using ICP-MS, 4.6 ± 3.20 µg/gm organ weight was detected in testes and 0.44 ± 0.18 µg/gm organ weight was detected in ovaries of treated groups (Table 1, Fig. 3). However, the pattern of accumulation was found to be nonsignificant when compared with the control group at a p-value > 0.05 on statistical analysis by analysis of variance (ANOVA) using Statistical Package for the Social Sciences (SPSS) software. Inductive coupled plasmon-mass spectroscopy is considered to be more sensitive instrument than ICP-AES as the detection limit for ICP-AES is 10 ppb while that for ICP-MS is 0.1 ppb.

Histopathological Analysis of the Gonads

Due to lack of reports available on histology of testes and ovaries in zebrafish, efforts were made to study the normal histological structure of testes and ovaries from the control group of male and female zebrafish respectively followed by histopathological analysis of the treated group of zebrafish males and females after subacute exposure to GNPs with an average size of 15 nm at a dose of 20 µg/gm. Histopathological observations for zebrafish testes were made under 100× objective while that for ovaries were made under 10× objective.

Germ Cells of Male Zebrafish

Testes are lateral, paired organs comprising of tubules or blind sacs, which are lined with spermatogenic epithelium. The seminiferous tubules are separated by thin strands of interstitial connective tissue containing Leydig cells, spermatogonia, spermatocytes, and spermatids.[23, 25] Testes of treated male zebrafish revealed the presence of testicular cells with typical architecture and normal spermatogenesis as evaluated by histology. Thus, no significant changes were observed when compared to control in testes (Figs 4A and B) on subacute exposure to GNPs for the given size at a given dose.

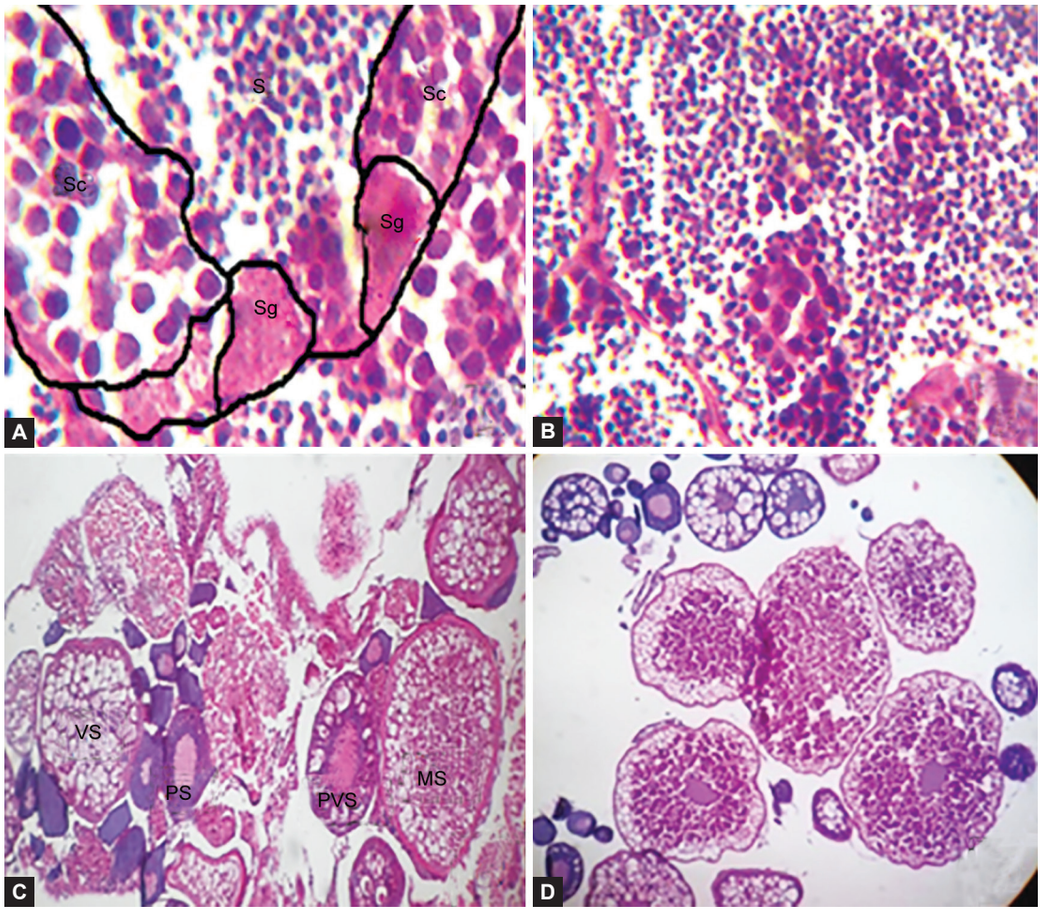
Germ Cells of Female Zebrafish

On examination of whole female gonads, the germinative parenchyma (epithelium) of the ovary showed a membrane-bound structure and constitutively contains oogonia, primary oocytes, pre (previtellogenic, vitellogenic, and mature oocytes) and postfollicular cells.[23,26] All features resembled that of the control and showed no significant changes in ovaries (Figs 4C and D) on subacute exposure to GNPs for the given size at a given dose.

DISCUSSION

This research was designed to explore the *in vivo* effects of GNPs in two types of zebrafish germinal cells; teticular and ovarian cells. So far, there have only been two studies published concerning the impact of GNPs on gametes. However, both trials concentrated on the male side, i.e., the effect of GNPs on spermatozoa. In each study, one uses chemically derived GNPs[27] while the other using laser-generated ligand-free particles.[28] Up to date there are no studies available concerning the impact of GNPs on oocytes.

In the present study, we were interested in exploring the toxic effects of GNPs on testicular and ovarian germ cells, in particular, their possible ability to cross physiological barriers and penetrate inside the germ cells, and in defining their localization. We choose zebrafish as a model mainly because of its biological resemblance to



Figs 4A to D: Histopathological analysis of testes (under 100× objective) and ovaries (under 10× objectives) from control and treated groups: (A) Histology of control male zebrafish showing different spermatogenic populations lined within the seminiferous tubules (Sg: Spermatogonia; Sc: Spermatocytes and S: Sperms), (B) histology of treated male zebrafish showing no changes in cellular morphology, (C) histology of control female zebrafish showing oocytes at different stages of development (PS: Primary oocyte stage; PVS: Previtellogenic stage; VS: Vitellogenic stage; MS: Mature stage), and (D) histology of treated female zebrafish showing no changes in cellular morphology

humans with respect to organ system homology and also due to the difficulty on working with higher vertebrate models.[29,30]

The present article is based on exposing adult male and female zebrafish toward GNPs with an average diameter of 15 nm via oral route and to investigate if gonads are the target for small nanoparticles. Also, study its interaction with both the types of germ cells. The results of this study demonstrate that though gold metal accumulation was detected in testes and ovaries after sub-acute exposure to 15 nm average sized spherical GNPs, it did not alter the cellular morphology of reproductive organs in zebrafish (*Danio rerio*) at a dose of 20 µg/gm. At this point, it cannot be considered as a reproductive toxicant at subacute exposures as there was no evident morphological disruption of germ cells in both males and females. The results of this study will be helpful for further research which can focus on the long-term effects induced by GNPs thus, setting standards for safety evaluation for metallic GNPs.

Although the present study gives a preliminary idea on germ cell response toward spherical GNPs of average size 15 nm, it can be suggested that GNPs of the selected size range used in this study seem to exert no negative effect on zebrafish germ cells, particularly on subacute exposure of 14 days. Further *in vivo* research should focus on prolonged exposure duration and possible genotoxicity of these GNPs on germinal cells to elucidate its effects and mechanism of action in human populations.

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ORIGINAL ARTICLE

Development of Regression Equation for the Measurement of Visceral Fat Area using Bioimpedance Technique

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ABSTRACT

Visceral fat is a predictor of obesity, metabolic syndrome and type II diabetes. The presently available technique, Computed tomography (CT) causes radiation exposure and is expensive. Abdominal obesity is a well established risk factor for obesity. There tends to be a risk of development of type 2 diabetes in obese individuals with abdominal obesity and insulin resistance. Abdominal obesity includes both subcutaneous and intra abdominal (visceral) adipose tissue and is associated with an increased risk of coronary heart disease (CHD) morbidity. A positive correlation between visceral fat, insulin levels and homeostasis model assessment insulin resistance index (HOMA-IR) in both genders was verified (r = 0.522 in boys and r = 0.309 in girls).

The study aims at developing a bioelectrical impedance based system for visceral fat area. The visceral fat area of 126 subjects (age: 38 ± 9 years) was first measured using the commercial instrument InBody 720 (Biospace, Korea) and then using the body composition analyzer (BCA) Bhabha Atomic Research Centre (BARC, Mumbai). Tetrapolar bioelectric impedance analysis (BIA) using two frequencies (50 KHz and 6.25 KHz) was used to develop the regression equation as follows: VFA = [–142 + 187* whr + 1.94* weight (Kg) + 0.135* Zbody 50 (Ω) + 1.027* age (years) –0.97* height (cm) + 7.2* sex –1.40* Zbody 50/W (Ω Kg^{-1})] cm^2; Sex = 0 for women and 1 for men (with R-sq adj = 96.87 and S = 5.37). The equation thus developed using BCA (BARC, Mumbai), validated with 60 subjects shows that there exists a high degree of correlation (R-sq adj = 96.87) between the two techniques.

Keywords: Bioelectrical impedance analysis, Biomedical instrumentation processor, Body composition analyzer, Visceral fat.

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INTRODUCTION

Obesity is a major concern today and is the cause of various diseases. Determination of visceral fat in the body helps to analyse obesity in individuals.

Visceral fat area (VFA) in the cross section of the abdomen is usually measured using computed tomography (CT).[1] Although CT scans provide an accurate assessment of the Visceral Fat, it has the disadvantages of being expensive and exposure of radiation to the patient. Thus there is a necessity to develop a cost effective and noninvasive system to evaluate VFA to be least cumbersome to the patient.

Bioelectrical impedance analysis (BIA) is a simple, noninvasive and cost effect technique to evaluate VFA.

In our experiment we adopted the tetra polar BIA technique. Tetrapolar technique involves injecting constant amplitude currents of two frequencies (50, 6.25 KHz) through two electrodes and measuring the voltage from the two other electrodes. This technique circumvents the error of inadvertent inclusion of electrode impedance.

The purpose of this research study is to develop and validate the developed regression equation for VFA using the body composition analyzer (BCA) developed (BARC, Mumbai) against InBody 720 (Biospace, Korea).

MATERIALS AND METHODS

The study subjects were 126 individuals (62 females and 64 males) between 19 and 60 years of age (38 ± 9 years). All the subjects were born and resided in India. The height and weights of all the individuals was measured before the test and they were informed about the procedure and the protocol. The height was estimated anthropometrically nearest to ± 1 cm. The measurements were made first by InBody 720 (Biospace, Korea) and then by the BCA developed in the laboratory (BARC, Mumbai). The data from the study subjects was collected before eating or 2 hours after eating.

Visceral fat area was obtained from the commercial instrument InBody 720 (Biospace, Korea) which automatically makes measurements at 6 different frequencies (1, 5,50,250,500,1000 KHz) incrementally, set

by the manufacturer. These frequencies are introduced into the body and the applied current ratings are 90 μA at 1 KHz and 400 μA at other frequencies. The product has been designed, manufactured and inspected under the full quality assurance of Biospace and fulfils the standards of IEC60601-1 and IEC 60601-2. The entire measurement takes around 5 to 6 minutes. The subjects are instructed to remove the watches and any other metallic objects. Subjects were asked to place their feet and heel on the two metallic electrodes provided at the base of the instrument and the palm and thumb on the handrails of the metallic grip electrodes. During the data acquisition it is required that the subjects do not flex and stand comfortably. The arms are required to be fully extended and at an angle of 15° from the trunk.

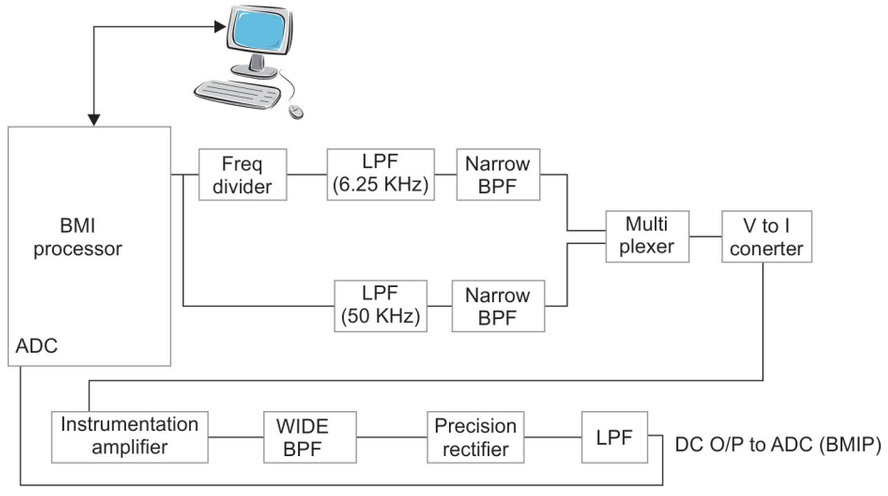
For measurements using BCA (BARC, Mumbai) the impedances at 50 and 6.25 KHz was measured for all the subjects. The instrument uses a whole body BIA approach. The subjects were asked to sit on a chair with their arms abducted to avoid touching the sides. Tetrapolar configuration was used in which braided copper wire electrodes (silver plated) were placed at the arm and foot. For both the programmed frequencies, an alternating current of less than 1 mA root means square is introduced into the body. The current injecting electrodes are placed on the palm and foot and voltage sensing electrodes are placed in the wrist and ankle. The right palm and foot were chosen as the electrode placement sites.

The values of the two impedances can be viewed at the user interface panel and the other already developed parameters will be displayed. The schematic block diagram used in the analysis is shown in Flow Chart 1. The biomedical instrumentation processor board (BMIPB) is used to initiate a communication between the PC and the developed system. The UART input clock is 36 MHz and by default serial port is set to 19200 baud. The entire system works at + 5V dc.

The BMI processor generates a square wave frequency of 50 KHz which is also given to a synchronous up/down counter. The synchronous up/down counter generates the second frequency of 6.25 KHz used in the design. Both the frequencies are then converted into pure sinusoidal alternating signals using a series of second order low pass filters and narrow band pass filters. The multiplexer selects any one frequency at a time. The selected frequency is passed through a V-I converter and fed to the patient through the isolation transformer. Through the voltage sensing electrodes, the developed voltage is amplified using a precision instrumentation amplifier and passed through a wide band pass filter to remove the superimposed noise and produce a pure sinusoidal alternating signal. Further, this output is also rectified and filtered to produce a pure DC voltage which is proportional to the impedance of the subject under investigation. This value is given to the ADC of the BMIPB and displayed on the user interface panel.

Using the impedances calculated at the 2 frequencies, additional BIA parameters like $Z_{body\ 50/6.25}/h$, $h^2/Z_{body\ 50/6.25}$, $w*h^2/Z_{body\ 50/6.25}$ were calculated. Stepwise regression was used to develop an equation for VFA. The level of significance was set at 0.15. All p-values less than 0.15 are considered. The BIA parameters with highest degree of correlation and least error were considered. The level of significance is the default value set by Minitab. Choosing a lesser value performs lesser steps in the stepwise regression analysis. Although selection of 0.05 level of significance yields the same results, the default value has been retained. The equation was developed and

Flow Chart 1: The body composition analyzer (BARC, Mumbai)



validated against a sample of 60 subjects. The correlation graphs and Bland Altman Plots were plotted.

This study cannot be performed with individuals having foot or leg amputations, pacemakers or implanted medical devices as it may cause the devices to malfunction.

RESULTS

A total of 126 individuals were subjected to this study. The anthropometric parameters of all the subjects are shown in Table 1. Stepwise regression was used for formation of the prediction equation shown below (with R-sq adj = 96.87 and S = 5.37). Figure 1 shows the correlation between BIA equation and the commercial instrument InBody 720 and Figure 2 shows the Bland Altman plots which was validated with a study group of 60 subjects.

The Bland Altman plot shows a bias of –0.176 denoted by the dark blue line. The bias denotes the mean difference between the commercial instrument InBody 720 and the developed BIA equation indicating the tendency to deviate from the true value.

The dashed blue lines indicate the 95% confidence interval of the bias.

The dashed red lines indicate the limits of agreement at ± 2 SD showing that VFA measured using the BIA equations will vary between +20.619 and –20.971.

The predicted equation for VFA is given as:

Visceral fat area = [–142 + 187* whr + 1.94* weight (kg) + 0.135* Zbody 50 (Ω) + 1.027* age (years) –0.97* height (cm) + 7.2* sex –1.40* Zbody 50/W(Ω kg^{-1})] cm^2; Sex = 0 for women and 1 for men.

Table 2 shows the stepwise regression performed with the software minitab. The constant has to be included in the equation. The first row of each predictor indicates the constant or the contribution of each predictor and has to be multiplied with each predictor. The t-value compares the means of the estimated and standard values. pvalue determines the appropriateness of rejecting the parameter in the equation. All p-values in this case are 0.000 < 0.15 and hence are accepted.

S denotes the standard error of the regression. R-sq (R^2) denotes the squared value of validity coefficient and R-sq (adj) is adjusted R-sq. The adjustment is important because the R^2 for any model will increase when a new term is added.

Table 1: Anthropometric characteristics of the study group

	Men	*Women*	*Combined*
Number	64	62	126
Age	37.25 ± 9.34	38.56 ± 9.47	37.89 ± 9.39
Weight	71.06 ± 11.05	60.59 ± 9.20	65.91 ± 11.42
Height	166.66 ± 6.74	155.72 ± 5.91	161.28 ± 8.37
BMI	25.49 ± 3	25.06 ± 4.04	25.28 ± 3.54

DISCUSSION

Studies have shown the relationship between obesity and several diseases in adult life, such as arterial hypertension, type 2 diabetes mellitus, cancer, and also cardiovascular mortality, body fat excess, mainly in the form of central obesity, is closely correlated with these diseases, mainly with nonalcoholic fatty liver disease (NAFLD).There is evidence to suggest that visceral adiposity is more influential than body mass in predicting fatty liver disease.[2] Abdominal obesity itself was found to be independently associated with an increased risk of coronary heart disease (CHD).[3]

Deposition of fat into ectopic areas depends on total body fat mass and an individual's basic susceptibility that is in turn related to age, gender, and ethnicity. Gender and ethnicity were found to influence group differences in visceral adiposity.[4]

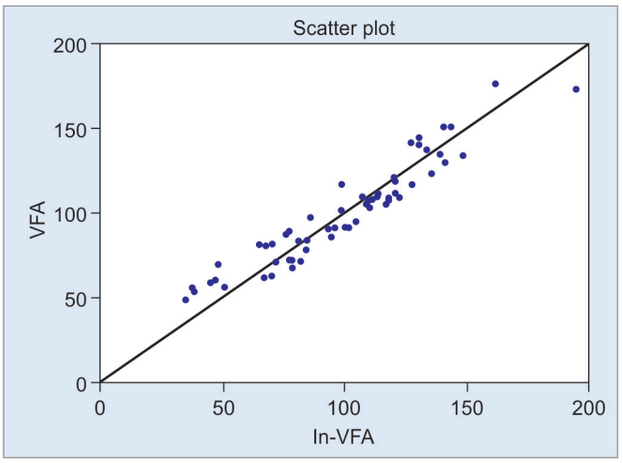


Fig. 1: Correlation graphs between InBody 720 (IN-VFA) and predicted values (VFA)

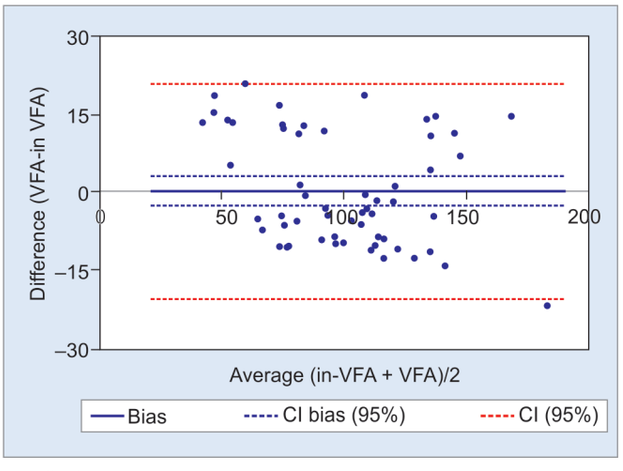


Fig. 2: Bland Altman plot for VFA: IN-VFA represents the data from commercial instrument InBody 720 (Biospace, Korea) and VFA represents the measurements using BCA (BARC, Mumbai). X-axis represents the mean (IN-VFA+VFA/2) and Y axis represents the difference (VFA-IN-VFA)

Table 2: Stepwise regression analysis

Response is VFA on 10 predictiors with n = 126			
Step	7	Height	– 0.97
Constant	– 142	t-value	– 5.54
whr	187	p-value	0.000
t-value	6.28		
p-value	0.000		
weight	1.94	Sex	7.2
t-value	12.66	t-value	3.45
p-value	0.000	S	5.37
Age	1.027	S	5.37
t-value	12.34	R-sq	97.05
p-value	0.000	R sq (adj)	96.87

Fat accumulation in the abdominal cavity increases intraabdominal pressure. The increase of intra-abdominal pressure observed in visceral obesity is able to pump upwards the diaphragmatic muscle, compressing the parenchyma of the lung, particularly at the basal regions. Moreover, the over-stretching of the diaphragmatic muscle fibers caused by the elevation of the diaphragmatic domes produced by visceral fat can decrease the contractile efficiency of the diaphragmatic muscle.[5]

Bioelectrical impedance analysis is a simple, noninvasive and cost effect technique to evaluate Visceral Fat Area. Bioimpedance refers to the electrical properties of a biological tissue, measured when current flows through it. In bioimpedance measurements, a current magnitude, about 800 μA, is chosen to be small enough so as not to be perceived by the subject, but large enough to produce voltages that are above interfering "noise".[6] It has the advantage of non exposure to radiation and ease of follow-up as compared to CT or DXA (Dual Energy X-ray absorptiometry).

The study shows that waist to hip ratio (WHR) acts as a positive predictor for visceral fat. Higher the WHR, higher is the visceral fat and *vice versa*. Visceral fat area at or below 100 cm^2 is considered normal whereas above 160 cm^2 is associated with high risk. It is also seen that higher the BMI higher is the value of VFA.

Regression analysis involves estimating statistical relationships between response variables and two or more predictor variables. In our study, regression equation for VFA is estimated. Minitab is software that assists in the analysis of data that is collected.

The commercial instrument InBody 720 uses segmental BIA approach showing impedance values for right arm, left arm, trunk, right leg, and left leg. For validation of commercial instrument InBody 720, Bone Mineral content of 22 healthy subjects was measured by InBody 720 and DEXA. High correlation (R = 0.9531) and low error (total error = 0.0913 kg) was found between these two methods.[7] The BCA developed uses full body BIA approach. The accuracy of the system can be improved by carefully measuring the height and through proper placement of the electrodes.

CONCLUSION

Bioelectric impedance analysis can be considered as a potential technology for measurement of different body composition parameters. Visceral fat area is one of the important parameters that can be determined by the instrument. The results of the analysis show that although there is a high degree of correlation between InBody 720 and the instrument developed, the standard error of regression (S = 5.37) remains high. Since Visceral fat is a deep seated fat located below the muscles, it would require higher frequencies for penetration. Use of frequencies in the range of 250 KHz would produce accurate results. The future implementation of the above said system will reduce the error and help in accurately determine VFA using BIA. The impedances for different individuals wary according to various factors like height, body weight, fat, and water in the body.

The analysis was carried out using Stepwise regression. Standard stepwise regression both adds and removes predictors as needed for each step. Minitab stops when all variables not in the model have pvalues that are greater than the specified alpha-to-enter value and when all variables in the model have pvalues that are less than or equal to the specified alpha-to-remove value. The shortcomings of stepwise regression are that if two predictor variables are highly correlated, only one might end up in the model even though either may be important. Stepwise regression might not always stop with the model with the highest R^2 value possible for a specified number of predictors.

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ORIGINAL ARTICLE

Geriatric Care: Are We Patient Friendly?

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ABSTRACT

Objectives: To study family satisfaction of medical care to geriatric patients and to find out any difference in family satisfaction given by geriatric specialist and general medicine specialist. Indian data on family's perception of geriatric care is lacking.

Materials and methods: All geriatric patients who were admitted between March 2015 and September 2015 in medical wards and where hospital stay was more than 3 days were included. An internationally validated family satisfaction questionnaire was used and administered to family member of each patient admitted. Scores were graded on Likert scale under five important areas like staff interaction, support services, quality of medical care, medical communication, and facilities. Special comments were analyzed separately.

Results: Patients' family members were most satisfied with quality of medical care and facilities (95%), followed by staff interaction and support facilities. Satisfaction regarding medical communication and services was poor (60%). There was no significant difference for satisfaction/perception of quality of care given by geriatric specialist and general medicine specialist.

Conclusion: Medical communication and support services of long-term care of geriatric patients are an important area of improvement. Family perception of geriatric care is vital to overall delivery of health care to this vulnerable group of patients. Support facilities specifically designed for geriatric patients need to be strengthened. There was no significant difference in perception of care offered by geriatric specialist and general medicine specialist.

Keywords: Family satisfaction, Geriatric care, Geriatric care specialist medicine.

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INTRODUCTION

Elderly or old age consists of aged nearing or surpassing the average life span of human beings. The boundary of old age cannot be defined exactly because it does not have the same meaning in all societies. Government of India adopted "National Policy on Older Persons" in January 1999. The policy defines "senior citizen" or "elderly" as a person who is of age 60 years or above. The elderly population which accounted for 6.7% of total population in 1991 is expected to increase its share to more than 10% by the year 2021. Among the states, the proportion of elderly in total population varies from around 4% in small states like Dadra and Nagar Haveli, Nagaland, Arunachal Pradesh, Meghalaya to more than 8% in Maharashtra, Tamil Nadu, Punjab, Himachal Pradesh, and 10.5% in Kerala in the recent census.[1]

The prevalence of disease in elderly is significant each year; around 5 million elderly patients are admitted to hospitals in India. Family members of elderly patients face unfamiliar stressful environment while they care for their elderly family member. High-quality medical care should be both patient and family centered. In our society, family support carries abundant significance. However, in reality, families' expectations and needs from health-care providers become secondary to the patients' medical care.[2]

Understanding and meeting the needs of the family members of the elderly patients is important. In the geriatric wards where the majority of patients are unable to participate in decision making about treatments, due to existing comorbidities (Fig. 1), the family's perspectives become central to understanding and measuring the satisfaction with the medical care provided.[3]

In the subset of elderly patients, studies on patients' family satisfaction are few in number and limited in scope. Culturally and socially, Indian families differ significantly as compared with those in the west; their expectations, needs, and factors contributing to their stress are likely to be different than those of the western

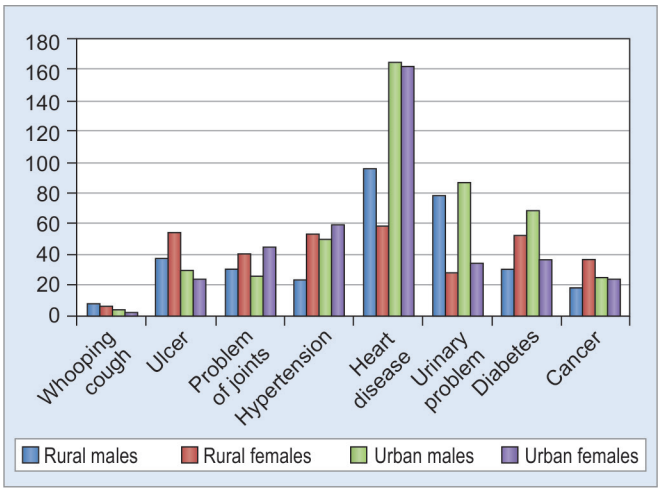

Fig. 1: Persons aged 60 years and above reporting a chronic disease (per 1,000 persons)

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families. Therefore, this study was conducted to assess family satisfaction of medical care given to geriatric patients and to assess whether it differed in families of patients admitted directly under geriatric specialist from those admitted under general medicine specialist.

AIMS AND OBJECTIVES

Aim

To study the family satisfaction of medical care given to geriatric patients at a tertiary care teaching hospital in Navi Mumbai.

Objectives

- To evaluate and determine the family satisfaction of medical care given to geriatric patient by using CARENET family satisfaction scale which was modified in order to meet the requirement of our study.[4] Permission for Scientific Committee was sought.
- To compare family satisfaction of medical care between geriatric consultant- and medical consultant-treated patients.

MATERIALS AND METHODS

Design of the study: This is a prospective, cross-sectional, observational, questionnaire-based survey. Family members of 200 consecutive geriatric patients fulfilling the inclusion criteria were enrolled.

Duration of the study: March 2015 to September 2015.

Setting: The study was conducted in a tertiary care teaching hospital in Navi Mumbai. The medical ward is a 180-bed, multidisciplinary ward, which admitted both medical and surgical patients. Physicians, who were not necessarily geriatric specialists, were also primarily responsible for the care of the elderly. The health-care team included the primary team admitting the patient from emergency department, a medicine department, and separate geriatric department along with bedside nurses and technicians with the average nurse-to-patient ratio 1:5. There is also separate rehabilitation department along with two patient/family counselor and a full-time dietician. Elderly patients are usually admitted in either medicine department or directly to geriatric department. Families were counseled by admitting physician. Families were allowed to be at the bedside when end-of-life care was being provided for terminally ill patients.

Inclusion: Patients of age more than 60 years who stayed in medical wards for more than 3 days were included in the study. The minimum stay of 3 days in the wards was chosen to ensure that the family member had adequate time and exposure to hospital environment. Only one family member in each patient's family – the key decision maker – was identified as spokesperson and was surveyed.

Exclusion: Patients less than 60 years were not included. Elderly patients admitted in the intensive care unit and those not willing for informed consent and participation were not included.

Sample size: We decided to include 200 consecutive patients arbitrarily, as we did not have any previous studies showing us a response rate or prevalence of specific variables.

Data collection: The questionnaire was administered on day 4 of the patient's hospital stay. Family members of geriatric patients were recruited consecutively, using the inclusion criteria. The questionnaire was administered in the privacy of special room in wards. All participants were specifically assured that their results would be kept confidential. For patients who stayed more than 3 weeks, the same questionnaire was administered on the 22nd day.

Survey questionnaire: An internationally reliable and validated family satisfaction scale[4] was adapted and modified to suit our setting. The questionnaire was administered. The questionnaire included the demographic details of patients such as age, gender, and date of admission, family members' relationship to patient (optional), physician under which the patient was admitted, and satisfaction scale items, which included self-rated levels of satisfaction with five identified key aspects of care related to the overall hospital experience like how the patient and the family member were treated, communication by the ward team, and support facilities. The survey consisted of 15 questions in five categories, patient quality care, medical communication, staff interaction, support services, and facilities. The answers were set to a Likert scale of 1–4, scoring was based on the scale, 1 denoting excellent/completely satisfied and 4 very poor/very dissatisfied. The space was provided for suggestions and comments (optional).

As the study was part of an ongoing quality improvement effort, ethical committee approval was not sought. The respondents were informed that participation was voluntary, and consent was implied by the completion of the survey.

Data analysis: Collected data for all the parameters were coded and analyzed with the statistical software Statistical Package for the Social Sciences (SPSS) 17.0 (SPSS IBM, USA). Descriptive statistics were calculated to describe the distributions of individual items and the summary scores. Means, medians, standard deviations, frequency tables, rates, and proportions were computed to describe the answers for each question and each category. Percentage of positive responses for each item was also computed. Answers that scored 3 and 4 were considered as a negative perception or not satisfactory. The scores were also standardized using the standardization formula

(Standardized Score = [Observed Score – Minimum Score]/[Maximum Score – Minimum Score]). The resultant scores in the scale of 0–100 were cut into halves using 50 as the midpoint. Chi-square tests were used to compare the satisfaction levels between families of patients admitted under geriatric specialist and general medicine specialist. t-tests were performed to compare the mean satisfaction before and after a long stay.

For all the statistical tests, $p < 0.05$ was considered as statistically significant.

RESULTS

A total of 200 family members of geriatric patients who stayed in the wards > 3 days were interviewed. One family member each for 200 patients completed the survey with 100% response rate. Of the 200 geriatric patients, 131 (65.5%) were males; 47 (23.5%) patients were admitted under geriatric specialist and 153 (76.5%) were admitted under general medicine specialist. Answers to individual questions were assessed, and proportions calculated, with higher scores indicating greater satisfaction (Fig. 2). The majority of respondents (189/200) were satisfied with overall care (95%).

The overall satisfaction response is summarized in Fig. 2. Families reported the greatest satisfaction with patient care (94.5%) and staff interaction (90.5%). They were less satisfied with the medical communication and support services (60.5%). Chi-square tests were performed for each of the five satisfaction domains to find out the association of satisfaction between geriatric specialist- and general medicine specialist-treated patients. The results revealed no statistically significant differences between both the groups (Table 1).

Satisfaction scores of patients admitted in the Department of Geriatrics and Medicine are shown in Tables 1 and 2 and Figures 2 and 3. Median scores were compared between geriatric and medical consultant. There were 7 patients who stayed in the wards for more than 3 weeks during the study period. There was no statistical significance between their "before and after" satisfaction scores.

Table 1: Comparative data showing the satisfaction of patients admitted in Departments of Geriatrics and Medicine

Family satisfaction domains		*Geriatric*	*Medicine*	*p-value**
Patient care	Satisfied	43	146	0.301
	Unsatisfied	4	7	
Staff interaction	Satisfied	45	140	0.334
	Unsatisfied	2	13	
Medical communication	Satisfied	43	144	0.523
	Unsatisfied	4	9	
Facilities	Satisfied	35	98	0.186
	Unsatisfied	12	55	
Support services	Satisfied	38	121	0.793
	Unsatisfied	9	32	

*p value obtained by using chi-square test

Table 2: Comparison of median scores: geriatrics and medicine

	Median score	
Category	*Geriatric consultant*	*Medicine consultant*
Patient care	4.0	4.0
Staff interaction	4.0	4.0
Medical communication	3.5	4.0
Facilities	3.5	4.0
Support services	3.0	3.0

Quantitative and Qualitative Data/ Written Comments

We analyzed the written comments, as they may add important insights not captured by the scores. More than half of the respondents in our survey provided comments; there were totally 103 comments (51.5%). Most comments related to communication with attendants

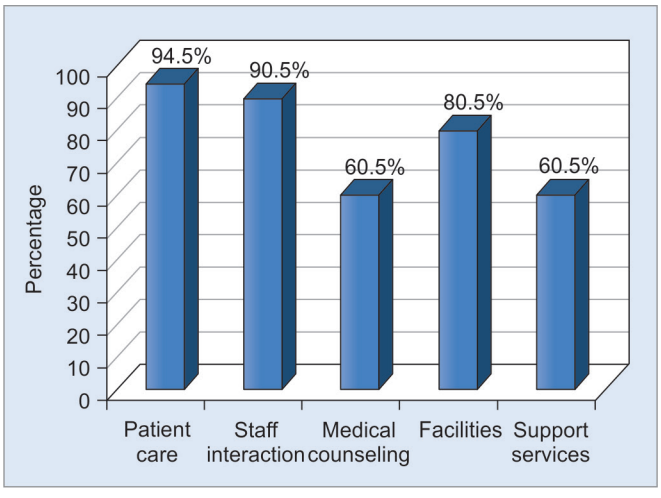

Fig. 2: The satisfactory response

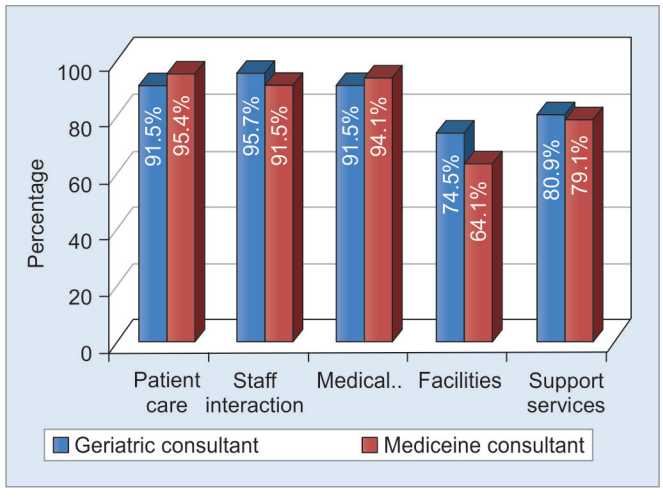

Fig. 3: The comparative satisfaction of patients admitted in Department of Geriatrics and Medicine

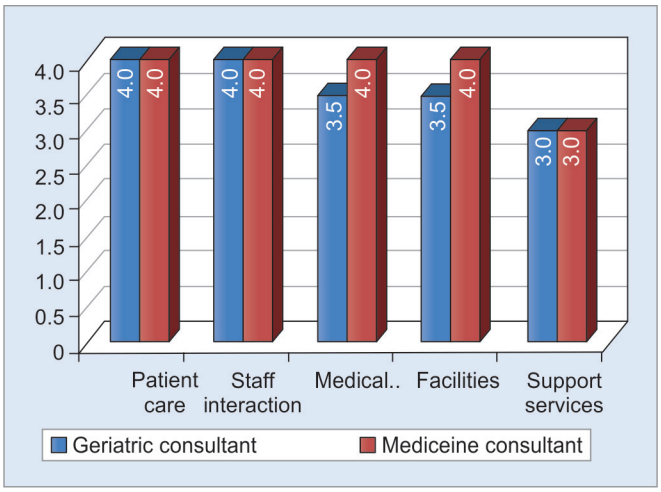

Fig. 4: Comparison of median scores between geriatrics and medicine consultant

followed by facilities provided for the families (rehabilitation measures, ambulation, speech, hearing, vision transportation for elderly in hospital). A total of 21 of the 103 comments (20.3%) were appreciations of overall care provided (Table 3). The number of positive and negative comments seemed to be in concordance with category-specific and overall satisfaction scores (Table 4). Most of the comments/suggestions were regarding communication being inadequate and ineffective.

DISCUSSION

Patient satisfaction is very much dependent on the interaction style and age of the patient. Mitchell Peck[5] found that understanding the factors and processes that determine patient satisfaction can ultimately significantly improve the many facets of health-care delivery. Sörensen et al[6] also consider the impact of individual differences when preparing the future care of adults. As matters stand presently, there is unfortunately a paucity of evidence on the effectiveness of end-of-life programs. Its effects on patients dying in an acute geriatric hospital setting are unknown.[7] Limited research funding leads to sparse and often contradictory palliative care and patient satisfaction literature, with few studies on causal mechanisms.[8] The key findings of our study suggest that overall the percentage of satisfaction was higher. Families reported greatest satisfaction with patient care (94.5%), staff interaction (90.5%), while being less satisfied with the medical communication and support services. This less satisfied domain needs to be further addressed. There have been a few studies that have attempted to research the expectations of geriatric patients in the developing world. Taimur Saleem et al's research in Pakistan attempted to find out the main causes of geriatric ill health and what produce high levels of patient satisfaction. Diabetes mellitus, hypertension, arthritis, and renal disease were common ailments among geriatric patients. However, it was clear that the patient satisfaction was mostly dependent on the way the prognosis is delivered by the physician: a realistic but optimistic picture was a certain way of achieving a high degree of patient satisfaction. Other factors that ensured a high degree of satisfaction was the opportunity to discuss treatment options, prescribing fewer rather than more medicine as well as the physician's overall knowledge of expertise in geriatric health.[9]

Also research in elderly care done by Sihame Lkhoyaali et al in Morocco focused on the role relatives play in caring for elderly cancer patients; 86% of relatives participated actively in the treatment making decisions of their elderly loved ones. However, the emotional cost of such care was high: 79.3% of relatives suffered from anxiety and 10% used anxiolytics; 38% also felt guilty for neglecting the early symptoms of their relatives. Depression and anxiety were more frequent among female relatives and among those of urban origin.[10] Another study in Sri Lanka looked at the challenges posed by the changing socioeconomic landscape and its impact on care for the elderly. Although both elders and caregivers still felt that elders should be looked after in the children's home, it was nonetheless clear that such an arrangement faced several challenges both from elders' viewpoint and the caregivers themselves. Elders feared losing their independence, while households where both the adult child and his/her spouse worked outside the home; households where elders had a disproportionate amount of household work; economically stressed households; and lack of direct communication between elders and caregivers also contribute to conflicts.[11] Another area that has been extensively researched is how the physician's personality can positively impact on patient care. Loyalty and trust in the physician's ability minimized patients.[12,13] A good physician–patient relationship increases patients' trust and willingness to communicate, so an awareness of the factors that influence this relationship is essential. Liang CY et al,[14] found that general practitioners could contribute massively to patients' well-being by helping older patients access health care and by extension improving the physician–patient relationship. Additional studies also found out that communication may best be achieved through efforts directed at those in earlier stages of the doctor–patient relationship.[15] Communication in the care of patients, with advanced and serious illness, can be improved using quality improvement interventions, particularly for health care utilization as an outcome. Interventions may be more effective using a consultative approach.[16] It has also been found that patient-centered communication is critical to quality cancer care in the

elderly as it helps patients and family members cope with cancer, make informed decisions, and effectively manage their care.[17]

However, balancing patients' best interests and the health-care scarce resources is a hard act at the best of times. Physicians often make nuanced trade-offs in clinical practice aimed at efficient resource use within a complex flow of clinical work and patient expectations. Understanding the challenges faced by physicians and the strategies they use to exercise cost-consciousness provides insight into policy measures that will address physician's roles in health care resource use.[18] Interestingly, the literature also suggests that patient-centered behavior was more dependent on the personal characteristics of the physician than the age, gender, dementia severity of the patients.[19] Another barrier identified to effective care was the lack of collaboration between health care professionals. Interprofessional work to deal with uncertainty and maintain coordinated care is needed for better palliative care provision to noncancer patients in the community. More research into development of a best model for effective interdisciplinary work is needed.[20] Ironically, more people die in hospital than in any other setting and it has been noted that care inputs operate in a mutually reinforcing manner to generate care outcomes, which implies that improvements in one area are likely to have spill-over effects in others.[21] Teamwork should be reinforced and actively encouraged.

CONCLUSION

Family members of elderly patients overall seem to be satisfied with our current services. There were no differences in family satisfaction whether the patients were admitted under geriatric specialist and medicine consultant. Family members expect better communication skills and rapport with the patients and relatives and involvement of family members in decision making. They also expect better support services for elderly patients and rehabilitation measures. Domains of low satisfaction provide a target to improve the quality of care both for the patients and their families.

IMPLICATIONS FOR PRACTICE

Training can be implemented to inform a team about the communication challenges, to equip them with effective communication skills, and improve their receptivity to patient cues. Information sharing can be used as a nonthreatening approach to initiate rapport-building and open communication. Team should consider patients' psychological readiness to communicate and respect their preference as to whom they wish to share their thoughts/emotions with. Hospitals/institutions also need to ensure a supportive ward culture and appropriate workload that will enable nurses to provide holistic care to patients.

Table 3: Positive and negative comments

Issues	*Number of suggestions*	*Positive*	*Negative*
Support services	23/103	34	66
Communication to attendants	11/103	88	12
Facilities	10/103	80	20

Table 4: Details of written comments

Categories	*Number of comments*
Support services	23
Appreciation	21
Communication to attendants	11
Facilities	10
Patient care	9
Satisfactory	7
Attendant care	7
Cost	6
Others	9
Total	103

IMPLICATIONS FOR RESEARCH

Further research on the effect of the Asian culture on effective communication within the ward setting is required to expand the knowledge in this area. Studies to ascertain the effect of the patients' age and place within the treatment cycle are also warranted. The lack of evidence on the effectiveness of postbasic communication education also requires further investigation.

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ORIGINAL ARTICLE

Blood Culture and Sensitivity Profile Study in a Tertiary Medical Hospital in Kolkata, West Bengal: 7 Years' Experience

[1]Ashis K Saha, [2]Kausik Munsi, [3]Payodhi Dhar

ABSTRACT

Aims and objectives: To identify the prevalence of bacteremia and the spectrum of antimicrobial sensitivity in our community, because it will guide the clinician to institute proper antimicrobial therapy.

Background: Bacteremia originates from either intravascular sites or extraVascular sites. In case of bloodstream infection, either Gram-positive or Gram-negative bacteria are responsible. Of these bacterial isolates, Gram-negative bacteria are responsible for higher mortality and morbidity. Since 20 to 30 years, coagulase-negative Staphylococci are responsible for most infection.

Materials and methods: In this retrospective study, blood samples were collected aseptically from 11,581 patients and were injected into the bottles containing bile-broth and brain-heart infusion broth and allowed to be incubated at 37°. Then subculture was done on blood agar, chocolate agar, as well as MacConkey agar media and was kept for 7 days or till the appearance of growth of the organism. After identification of isolates, Kirby Bauer disk diffusion test on Mueller-Hinton agar II was performed to detect antimicrobial sensitivity.

Results: Our study documented 8.58% positive cultures in the last 7 years. Gram-negative bacterial isolates were significantly higher than Gram-positive isolates (64.19% *vs* 34.80%, p = 0.00). Lowest number of positivity was seen in *Morganella* (0.40%) followed by *Proteus* (0.50%) and *Enterococcus faecium* (0.90%) in ascending order. Males were significantly more culture positive than females (549/994 *vs* 445/994, p = 0.00). Most common bacterial isolates were (coagulase negative Staphylococci) CoNS (239, 24.04%) followed by *Klebsiella* including ESBL (extended spectrum beta-lactamase), carbapenamase producer (234, 23.74%) and *Escherichia coli* (110, 11.06%). *E. coli* was >75% sensitive to imipenem group, polymyxin B (98.18%), colistin (96.36%), and amikacin (80.9%). Coagulase negative *Staphylococci* showed more than 60% sensitivity to levofloxacin (76.98%), amikacin (82.84%), tigecycline (87.44%), vancomycin (94.45%), teicoplanin (91.63%), linezolid (91.21%), gentamicin (76.56%), netilmicin (74.47%), and tetracycline (75.31%). *Klebsiella* (non-ESBL and carbapenemase producer) was highly sensitive to polymyxin B (93.06%), colistin (91.90%), meropenem (65.31%), and imipenem (94.73%). Extended spectrum beta-lactamase-producing *Klebsiella* showed increased sensitivity to meropenem (89.47%), imipenem (94.73%), ertapenem (81.57%), polymyxin B, and colistin (97.36% each).

Conclusion: Positive cultures were 8.58% in the last 7 years. Gram negative bacterial isolates were significantly higher. Males were more culture positive. Most common bacterial isolates were CoNS followed by *Klebsiella* species and *E. coli*. Gram-negative bacterial isolates were highly sensitive to piperacillin, cefoperazone, imipenem, meropenem, aminoglycoside group of antibiotics, tigecycline, polymyxin B and colistin. Gram-positive bacterial isolates were sensitive to piperacillin, cefoparazone, vancomycin, teicoplanin, linezolid and clindamycin. *Salmonella typhi* were sensitive to ampicillin, cefoparazone, cefepime, azithromycin, chloramphenicol, and fluoroquinolones. *Acinetobacter* showed >50% sensitivity to cefepime and *Pseudomonas* showed >50% sensitivity to cefotaxime and levofloxacin. So to prevent resistance of bacterial isolates, a proper antibiotic guideline should be maintained.

Keywords: Blood culture and sensitivity, Coagulase negative *Staphylococcus*, *E. coli*, Gram-positive and Gram-negative bacterial isolates, *Klebsiella*, Tertiary care hospital.

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INTRODUCTION

Episodic bacterial colonization in blood circulation is called bacteremia.[1] This bloodstream infection may be life threatening in few circumstances leading to septic shock and eventually death. Bloodborne infection is a major cause of morbidity and mortality.[2] In 1899, Brill reported the first case of bacteremia due to bacillus pyocyaneus (which is now known as *Pseudomonas aeruginosa*). After 10 years, 40 cases were reported worldwide and 30 more cases were documented in the next 15 years.[3] There are two types of bloodstream infections: first one is intravascular – it originates from cardiovascular system, e.g., infective endocarditis, mycotic aneurysm, catheter insertion. Second one is extravascular – it originates from extravascular sites, like lung, lymphatic system, kidney, bones, etc.[4] Malaria and bloodstream infection are clinically indistinguishable when a patient comes with fever,

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even the World Health Organization has failed to identify and distinguish these two cases.[5-8] In case of bacterial infection, Gram-negative bacteria are responsible for more mortality than infection due to Gram-positive bacteria. Over the past 20 to 30 years, *Escherichia coli* and staphylococcal infection continue to be the most common causative organisms, but recently, bacterial spectrum has changed. Nowadays, there is substantial increase in bloodstream infection due to coagulase negative *Staphylococcus*.[9] *Staphylococcus epidemidis* is responsible for infection in pediatric population followed by incidence of *Staphylococcus aureus* infection. There is also increasing frequency of changes in antimicrobial resistance of microbial isolates throughout the world.[10,11] For example, there is emerging resistance to fluoroquinolones and broad spectrum cephalosporins in Enterobacteriaceae, oxacillin in Staphylococci, vancomycin in Enterococci, and penicillin in Streptococci.[10,12] So, to prevent mortality, rapid and reliable detection of bacteria is essential worldwide and it can be accomplished by doing blood culture in proper time and in proper condition. This will guide the physicians to introduce proper antibiotic in adequate doses. In this study, our aim was (1) to identify the prevalence of microbes in our patients, (2) to study the spectrum of antimicrobial sensitivity to these microbes by performing *in vitro* culture sensitivity test, because it will guide the clinician to institute the proper antimicrobial therapy.[13]

MATERIALS AND METHODS

This was a retrospective study of 7 years period (2009 to 2015) in KPC Medical College and Hospital, Kolkata, West Bengal. This study was started only after getting permission from our local ethical committee.

Study Populations

Patients with respiratory tract infection, urinary tract infection, infective endocarditis, large abscesses in different areas of the body, osteomyelitis, ventilator-associated pneumonia, catheter-induced infection were recruited for the study.

Sample Size

A total of 11,581 blood cultures were performed, of which 994 cultures were positive.

Method of Culture

Blood samples were collected aseptically using sterile needle and syringe from the patients before antibiotic administration. But in case of patients getting antibiotics, prior to next schedule dose blood sample was collected. In case of adult and pediatric patients, 5 and 2 ml blood was collected respectively.[4]

Processing of Specimen

Aseptically collected blood was injected into the bottles containing bile broth and brain-heart infusion broth and allowed to be incubated at 37°. Blood bottles were examined periodically at regular intervals for any evidence of bacterial growth, hemolysis, turbidity, and any gas production. Then subculture was done on blood agar, chocolate agar, as well as MacConkey agar media and was kept for 7 days or till the appearance of growth of the organism.[14] Then the colonies were processed for a series of tests for identification of isolates based on motility test, Gram stain, and biochemical tests. After identifying the isolates, Kirby Bauer disk diffusion test on Mueller-Hinton agar II was performed to detect antimicrobial sensitivity according to recommendation of Clinical and Laboratory Standard Institute.[15] In each culture plate, antibiotic disk (oxoid) was applied and kept for 24 hours in 35°C. Antibiotic zones were measured and interpreted accordingly.

Statistical Analysis

The data were analyzed by using software Statistical Package for the Social Sciences (SPSS) version 18. A value of $p < 0.05$ was accepted as significant.

RESULTS

Total number of positive cultures was 994 out of total blood sent for culture of 11,581, percentage of positivity being 8.58%. Among the 7 years' study, in 2014 and 2015, the percentages of positive blood cultures were highest (17–16.49% respectively). Again in these 2 years, males showed significantly positive cultures as compared with females (102 *vs* 67 in 2014 and 95 *vs* 69 in 2015, $p = 0.00$). Similar result was demonstrated in 2011 (89 in males *vs* 46 in females, $p = 0.00$). Again, in total study, males were significantly positive than females (549 *vs* 445, $p = 0.00$) (Table 1).

Table 1: Year-wise male and female distribution of bacterial isolates

		Sex		
Years	*Total cases (994)*	*Males*	*Females*	*p-value*
2009	105 (10.56%)	53	52	0.89
2010	129 (12.97%)	71	58	0.10
2011	135 (13.58%)	89	46	0.00
2012	151 (15.19%)	74	77	0.72
2013	141 (14.18%)	65	76	0.19
2014	169 (17%)	102	67	0.00
2015	164 (16.49%)	95	69	0.00
		549	445	0.00

Table 2: Sex-wise distribution of bacterial isolates

Bacterial isolates	*Males*	*Percentage*	*Females*	*Percentage*	*p-value*
Escherichia coli (110) (11.06%)	54	49.09	56	50.90	0.78
Coagulase negative *Staphylococcus* (239) (24.04%)	120	43.93	119	49.79	0.92
Klebsiella (173) (17.40%)	105	60.69	68	39.30	0.00
Klebsiella (ESBL) (38) (3.82%)	25	65.57	13	34.21	0.00
Klebsiella (CARB) (25) (2.51%)	15	60	10	40	0.15
Morganella (4) (0.40%)	3	75	1	25	0.15
Proteus (5) (0.50%)	2	40	3	60	0.52
Enterococcus faecium (9) (0.90%)	6	66.66	3	33.33	0.15
Staphylococcus (86) (8.65%)	45	52.32	41	47.67	0.54
Citrobacter (22) (2.21%)	14	63.63	8	36.36	0.07
Nonfermenting Gram negative bacilli (73) (7.34%)	49	67.12	24	32.87	0.00
Enterococcus (12) (1.20%)	6	50	6	50	1
Acinetobacter (58) (5.83%)	26	44.82	32	55.17	0.26
Pseudomonas (75) (7.54%)	37	49.33	38	50.66	0.47
Salmonella (41) (4.12%)	28	68.29	13	31.70	0.00
Burkholderia cepacia (24) (2.41%)	14	58.33	10	41.66	0.24

ESBL: Extended spectrum beta-lactamase

In this study, percentage of positive cultures was highest in coagulase negative *Staphylococcus* (CoNS) (24.04%) cases, followed by *Klebsiella* (17.04%) and *E. coli* (11.06%), whereas lowest number of positivity was seen in *Morganella* (0.40%) followed by *Proteus* (0.50%) and *Enterococcus faecium* (0.90%) in ascending order. Again in case of culture positivity of *Klebsiella*, *Klebsiella* (extended spectrum beta-lactamase, ESBL producer), nonfermenting Gram-negative bacilli, and *Salmonella typhi* incidence of males were significant than in females (105 *vs* 68, 25 *vs* 13, 49 *vs* 24, and 28 *vs* 13 respectively, p = 0.00) (Table 2). In our study, the total number of Gram-positive bacteria was 346 and Gram-negative bacteria was 644, with p-value of 0.00 (Table 3).

Table 3: Total Gram-positive and negative bacteria

Gram-positive bacteria	*Gram-negative bacteria*	
Coagulase negative *Staphylococcus* (239)	*E. coli* (110)	p-value
Enterococcus faecium (9)	*Klebsiella* (173)	
Staphylococcus (86)	*Klebsiella* (ESBL) (38)	
Enterococcus (12)	*Klebsiella* (CARB) (25)	
	Proteus (5)	
	Citrobacter (22)	
	Nonfermenting Gram negative bacilli (73)	
	Acinetobacter (58)	
	Pseudomonas (75)	
	Salmonella (41)	
	Burkholderia cepacia (24)	
	Morganella (4)	
Total = 346	Total = 648	0.00

ESBL: Extended spectrum beta-lactamase

Escherichia coli was > 75% positive to imipenem group, polymyxin B (98.18%), colistin (96.36%), amikacin (80.9%), and between 60 and 74% to other aminoglycoside group of drugs and piperacillin (69.09%). Coagulase negative Staphylococci showed more than 60% positivity to levofloxacin (76.98%), amikacin (82.84%), tigecycline (87.44%), vancomycin (94.45%), teicoplanin (91.63%), linezolid (91.21%), gentamicin (76.56%), netilmicin (74.47%), and tetracycline (75.31%). *Klebsiella* (non-ESBL and carbapenemase producer) was highly sensitive to polymyxin B (93.06%), colistin (91.90%), meropenem (65.31%), and imipenem (94.73%), whereas ESBL-producing *Klebsiella* showed increased sensitivity to meropenem (89.47%), imipenem (94.73%), ertapenem (81.57%), with highest positivity to polymyxin B and colistin (97.36% each). But carbapenemase-producing Klebsiella was 100% positive only to polymyxin B and colistin. *Staphylococcus aureus* was highly sensitive to vancomycin (96.51%), linezolid (97.67%), teicoplanin (89.53%), clindamycin (82.55%), tetracycline (82.55%), gentamicin (80.23%) and 100% resistant to polymyxin B and colistin. *Acinetobacter* showed high sensitivity to only polymyxin B (84.48%) and colistin (82.75%), meropenem (62.06%), and imipenem (62.06%). *Pseudomonas* bacteria showed high sensitivity to piperacillin (80%), cefoperazone (66.68%), imipenem (92%), meropenem (70%), gentamicin (78.66%), amikacin (81.33%), netilmicin and tobramycin (76% each), fluoroquinolones (ciprofloxacin 76%, levofloxacin 89.33%), polymyxin B (94.66%), and colistin (92%). Lastly, *Salmonella typhi* was highly sensitive to chloramphenicol (73.17%), ciprofloxacin (73.1%), levofloxacin (75.60%), imipenem (90.24%), ceftriaxone (32.68%), piperacillin (75.60%), and cefotaxime (73.17%) (Tables 4A to D).

Table 4A: Antibiotic sensitivity of bacterial isolates

Organism	*PEN*	*AMOX*	*OX*	*AMC*	*PIP*	*CES*	*CEFU*	*CFT*	*TIC*
Escherichia coli (110)	0	4 (3.63%)	1 (0.9%)	26 (23.63%)	76 (69.09%)	64 (58.18%)	12 (10.9%)	12 (10.9%)	3 (2.72%)
CoNS (239)	4 (1.67%)	20 (8.36%)	77 (32.21%)	111 (46.44%)	156 (65.27%)	10 (4.18%)	48 (20.08%)	4 (1.67%)	5 (2.09%)
Klebsiella (173)	0	5 (2.89%)	2 (1.15%)	25 (14.45%)	69 (39.88%)	57 (32.94%)	11 (6.35%)	30 (17.34%)	10 (5.78%)
Klebsiella (ESBL) (38)	0	0	0	0	18 (47.36%)	15 (39.47%)	0	0	0
Klebsiella (CARB) (25)	0	0	0	0	0	0	0	0	0
Morganella (4)	0	0	0	0	3 (1.2%)	1 (4%)	0	1 (4%)	0
Proteus (5)	0	0	0	0	2 (40%)	4 (80%)	0	0	0
Enterococcus faecium (9)	0	1 (11.11%)	0	2 (22.22%)	2 (22.22%)	1 (11.11%)	0	1 (11.11%)	1 (11.11%)
Staphylococcus (86)	6 (6.97%)	23 (26.74%)	48 (55.81%)	53 (61.62%)	57 (66.27%)	4 (4.65%)	33 (35.37%)	14 (16.27%)	1 (1.16%)
Citrobacter (22)	0	3 (13.63%)	1 (4.54%)	4 (18.18%)	16 (72.72%)	12 (54.54%)	3 (13.63%)	7 (31.81%)	5 (22.72%)
Nonfermenting Gram negative bacilli (73)	0	2 (2.73%)	1 (1.36%)	4 (5.47%)	62 (84.93%)	47 (64.38%)	3 (4.10%)	6 (82.19%)	7 (9.58%)
Enterococcus (12)	0	3 (25%)	0	8 (66.66%)	9 (75%)	1 (8.33%)	1 (8.33%)	1 (8.33%)	1 (8.33%)
Acinetobacter (58)	0	1 (1.72%)	0	6 (10.34%)	22 (37.93%)	16 (27.58%)	3 (5.17%)	7 (12.06%)	9 (15.51%)
Pseudomonas (75)	0	0	0	2 (2.66%)	60 (80%)	50 (66.66%)	0	4 (53.33%)	11 (14.66%)
Salmonella (41)	0	5 (12.19%)	0	24 (58.53%)	31 (75.60%)	24 (58.53%)	2 (4.87%)	30 (73.17%)	9 (21.95%)
Burkholderia cepacia (24)	0	1 (4.16%)	0	1 (4.16%)	20 (83.33%)	9 (37.5%)	1 (4.16%)	2 (8.33%)	0

Table 4B: Antibiotic sensitivity of bacterial isolates

Organism	*CXT*	*CFZ*	*CTRX*	*CFP*	*AZ*	*ER*	*AZT*	*ERT*	*IMP*	*CEFO*
E. coli (110)	46 (41.18%)	22 (20%)	26 (23.63%)	22 (20%)	5 (4.54%)	0	17 (18.70%)	83 (75.45%)	98 (89.09%)	0
CoNS (239)	52 (21.75%)	6 (2.51%)	76 (31.79%)	36 (15.06%)	52 (21.75%)	80 (33.47%)	38 (15.89%)	37 (15.48%)	104 (43.51%)	2 (0.83%)
Klebsiella (173)	58 (33.52%)	20 (11.56%)	31 (17.91%)	25 (14.45%)	15 (8.67%)	0	16 (9.24%)	102 (58.95%)	118 (68.20%)	2 (1.15%)
Klebsiella (ESBL) (38)	25 (65.78%)	0	0	0	0	0	0	31 (81.57%)	36 (94.73%)	0
Klebsiella (CARB) (25)	0	0	0	0	0	0	0	0	0	0
Morganella (4)	0	2 (50%)	1 (25%)	0	0	0	0	1 (25%)	3 (75%)	0
Proteus (5)	1 (20%)	0	0	0	0	0	0	4 (80%)	4 (80%)	0
Enterococcus faecium (9)	1 (11.11%	1 (11.11%)	1 (11.11%)	0	1 (11.11%)	0	0	1 (11.11%)	2 (22.22%)	0
Staphylococcus (86)	14 (16.27%)	1 (1.16%)	55 (63.95%)	38 (44.18%)	31 (36.04%)	55 (63.95%)	26 (30.23%)	16 (18.60%)	21 (24.41%)	1 (1.16%)
Citrobacter (22)	5 (22.72%)	4 (18.18%)	6 (27.27%)	3 (13.63%)	2 (9.09%)	1 (4.54%)	4 (18.18%)	20 (90.90%)	21 (95.45%)	0
Nonfermenting Gram negative bacilli (73)	3 (4.10%)	7 (9.58%)	11 (15.06%)	8 (10.95%)	9 (12.32%)	1 (1.36%)	4 (5.47%)	23 (31.50%)	68 (33.15%)	1 (1.36%)
Enterococcus (12)	0	2 (16.66%)	2 (16.66%)	1 (8.33%)	2 (16.68%)	5 (41.66%)	4 (33.33%)	3 (25%)	9 (75%)	0
Acinetobacter (58)	3 (5.17%)	7 (12.06%)	10 (17.24%)	5 (86.20%)	7 (12.06%)	0	2 (3.44%)	13 (22.41%)	36 (62.06%)	1 (1.72%)
Pseudomonas (75)	1 (1.33%)	28 (37.33%)	3 (4%)	27 (36%)	5 (6.66%)	0	24 (32%)	8 (10.66%)	69 (92%)	25 (33.33%)
Salmonella (41)	3 (7.31%)	12 (29.26%)	38 (32.68%)	23 (56.09%)	26 (63.41%)	0	19 (46.34%)	13 (31.70%)	37 (90.24%)	2 (4.87%)
Burkholderia cepacia (24)	1 (4.16%)	3 (12.5%)	2 (8.33%)	5 (20.88%)	2 (8.33%)	0	4 (16.66%)	5 (20.88%)	16 (66.66%)	0

Table 4C: Antibiotic sensitivity of bacterial isolates

Organism	*MER*	*GEN*	*TOB*	*NET*	*AMK*	*NLX*	*NF*	*CIP*	*OF*	*LEV*
E. coli (110)	95 (86.36%)	76 (69.09%)	74 (67.27%)	82 (74.54%)	89 (80.90%)	0	0	40 (36.36%)	36 (32.72%)	56 (50.90%)
CoNS (239)	61 (25.52%)	183 (76.56%)	98 (41%)	178 (74.47%)	198 (82.84%)	0	10 (4.18%)	134 (56.06%)	140 (58.57%)	184 (76.98%)
Klebsiella (173)	113 (65.31%)	78 (45.08%)	77 (44.50%)	70 (40.46%)	88 (50.86%)	0	0	50 (28.90%)	42 (24.27%)	66 (38.15%)
Klebsiella (ESBL) (38)	34 (89.47%)	16 (42.10%)	17 (44.73%)	18 (47.36%)	23 (60.52%)	0	0	11 (28.94%)	9 (23.68%)	14 (36.84%)
Klebsiella (CARB) (25)	0	1 (4%)	1 (4%)	1 (4%)	1 (4%)	0	0	0	0	0
Morganella (4)	2 (50%)	2 (50%)	2 (50%)	2 (50%)	2 (50%)	0	0	3	0	3 (75%)
Proteus (5)	4 (80%)	2 (40%)	2 (40%)	2 (40%)	2 (40%)	0	0	0	2 (40%)	2 (40%)
Enterococcus faecium (9)	1 (11.11%)	1 (11.11%)	0	0	1 (11.11%)	0	0	0	0	0
Staphylococcus (86)	17 (19.76%)	69 (80.23%)	18 (20.93%)	61 (70.93%)	64 (74.41%)	0	0	59 (68.60%)	59 (68.60%)	64 (74.41%)
Citrobacter (22)	19 (86.36%)	13 (59.09%)	14 (63.63%)	13 (59.09%)	15 (68.18%)	0	0	11 (50%)	11 (50%)	18 (81.81%)
Nonfermenting Gram negative bacilli (73)	67 (91.78%)	46 (63.01%)	43 (58.90%)	46 (63.01%)	49 (67.12%)	3 (4.10%)	0	54 (73.97%)	42 (57.53%)	62 (84.93%)
Enterococcus (12)	2 (16.66%)	8 (66.66%)	2 (16.66%)	1 (8.33%)	2 (16.66%)	0	0	8 (66.66%)	3 (25%)	8 (66.66%)
Acinetobacter (58)	36 (62.06%)	21 (36.20%)	19 (32.75%)	19 (32.75%)	22 (37.93%)	0	0	23 (39.65%)	7 (12.06%)	31 (53.44%)
Pseudomonas (75)	70 (93.33%)	59 (78.66%)	57 (76%)	57 (76%)	61 (81.33%)	0	1 (1.33%)	57 (76%)	37 (49.33%)	67 (89.33%)
Salmonella (41)	28 (68.29%)	4 (9.75%)	3 (7.31%)	3 (7.31%)	3 (7.31%)	17 (41.46%)	1 (2.43%)	30 (73.17%)	22 (53.65%)	31 (75.60%)
Burkholderia cepacia (24)	16 (66.66%)	9 (37.5%)	7 (29.16%)	7 (29.16%)	8 (33.33%)	0	0	15 (62.5%)	3 (12.5%)	17 (70.83%)

Table 4D: Antibiotic sensitivity of bacterial isolates

Organism	*COT*	*CHLO*	*NIF*	*TET*	*TIG*	*CLIN*	*VAN*	*TEI*	*LID*	*POL*	*COL*
E. coli (110)	50 (45.45%)	55 (50%)	3 (2.72%)	38 (34.45%)	87 (79.90%)	0	0	0	0	108 (98.18%)	106 (96.36%)
CoNS (239)	116 (48.53%)	170 (71.12%)	0	180 (75.31%)	209 (87.44%)	153 (64.01%)	226 (94.45%)	219 (91.63%)	218 (91.21%)	11 (4.60%)	11 (4.60%)
Klebsiella (173)	62 (35.83%)	67 (38.72%)	0	73 (42.19%)	134 (77.45%)	1 (0.57%)	0	2 (1.15%)	1 (0.57%)	161 (93.06%)	159 (91.90%)
Klebsiella (ESBL) (38)	15 (39.47%)	21 (55.26%)	0	18 (47.36%)	32 (84.21%)	0	0	0	0	37 (97.36%)	37 (97.36%)
Klebsiella (CARB) (25)	1 (4%)	3 (12%)	0	10 (40%)	21 (84%)	0	0	0	0	25 (100%)	25 (100%)
Morganella (4)	3 (75%)	1 (25%)	0	3 (75%)	3 (75%)	0	0	0	0	1 (25%)	1 (25%)
Proteus (5)	0	3 (60%)	0	0	0	0	0	0	0	0	0
Enterococcus faecium (9)	1 (11.11%)	6 (66.66%)	0	5 (55.55%)	6 (66.66%)	0	7 (77.77%)	7 (77.77%)	7 (77.77%)	1 (11.11%)	1 (11.11%)
Staphylococcus (86)	54 (62.79%)	59 (68.60%)	1 (1.15%)	71 (82.55%)	60 (69.76%)	71 (82.55%)	83 (96.51%)	77 (89.53%)	84 (97.67%)	0	0
Citrobacter (22)	7 (31.81%)	13 (59.09%)	1 (4.54%)	12 (54.54%)	17 (77.27%)	0	0	0	0	22 (100%)	22 (100%)
Nonfermenting Gram negative bacilli (73)	55 (75.34%)	12 (16.43%)	0	24 (32.87%)	26 (35.61%)	2 (2.73%)	3 (4.10%)	2 (2.73%)	3 (4.10%)	20 (27.39%)	22 (30.13%)
Enterococcus (12)	2 (16.66%)	4 (33.33%)	0	5 (41.66%)	3 (25%)	1 (8.33%)	8 (66.66%)	7 (58.33%)	8 (66.66%)	3 (25%)	3 (25%)
Acinetobacter (58)	20 (34.48%)	14 (24.13%)	2 (3.44%)	22 (37.93%)	35 (60.34%)	0	0	0	0	49 (84.48%)	48 (82.75%)
Pseudomonas (75)	12 (16%)	3 (4%)	0	4 (5.33%)	6 (8%)	0	0	0	0	71 (94.66%)	69 (92%)
Salmonella (41)	22 (53.65%)	30 (73.17%)	0	12 (29.26%)	8 (19.51%)	1 (2.43%)	1 (2.43%)	0	1 (2.43%)	16 (39.02%)	16 (39.02%)
Burkholderia cepacia (24)	18 (75%)	3 (12.5%)	0	4 (16.66%)	7 (29.16%)	0	0	0	0	1 (4.16%)	1 (4.16%)

CoNS: Coagulase negative *Staphylococcus*; ESBL: Extended spectrum beta-lactamase; PEN: Penicillin; AMOX: Amoxycillin; OX: Oxacillin; AMC: Ampicillin; PIP: Piperacillin; CES: Cefoperazone + Sulbactum; CEFU: Cefuroxime; CFT: Cefotaxime; TIC: Ticarcillin; CXT: Cefoxitin; CFZ: Ceftazedime; CTRX: Ceftriaxone; CFP: Cefepime; AZ: Azithromycin; ER: Erythromycin; AZT: Aztreonum; IMP: Imipenem; CEFO: Cefoperazone; MER: Meropenem; GEN: Gentamicin; TOB: Tobramycin; NET: Netelmycin; AMK: Amikacin; NLX: Nalidixic acid; NF: Norfloxacin; CIP: Ciprofloxacin; OF: Ofloxacin; LEV: Levofloxacin; COT: Cotrimoxazole; CHLO: Chloramphenicol; NIF: Nitrofurantoin; TET: Tetracycline; TIG: Tigicycline; CLIN: Clindamycin; VAN: Vancomycin; TEI: Teicoplanin; LID: Linezolid; POL: Polymyxin; COL: Colistin

DISCUSSION

Our study revealed blood culture sensitivity of 8.58%, which was nearly half of the value as shown in the studies of Mehta et al (16.8%),[16] Qureshi and Aziz (16.6%),[17] and Devi et al (16.8%).[18] This low rate of positivity may be due to reasons like proper clinical care, proper antibiotic administration prior to admission in the tertiary care hospital, and most importantly prohibition over-the-counter antibiotic therapy.

In our study, incidence of Gram negative isolates was significantly high as compared with Gram-positive isolates (648, 65.19% *vs* 346, 34.80%, p = 0.00). It is compared with the study done by Irenge Leonid et al (Gram-negative isolate 104, 61.2% *vs* Gram-positive isolates 66, 38.8%, p = 00).[19] But on the contrary, study done by Moyo et al as well as studies done in Tanzania and other African countries showed prevalence of Gram-positive bacterial isolates over Gram-negative isolates (82% Gram-positive *vs* 17.9% Gram-negative bacteria).[9,20,21] Other studies in the developing countries similarly showed significant prevalence of Gram-positive bacterial isolates in bloodstream infections.[8,9,22,23]

There was no significant difference in gender involvement with respect to bacterial isolates in the studies done by Jombo et al (89/1260 *vs* 111/1910, p > 0.05)[24] and Devi et al (males *vs* females = 48.93% *vs* 51.06%, p > 0.05).[18] But in our study males showed significant positive bacterial isolates as compared with females (549/994 *vs* 445/994, p = 0.00).

Our study showed the most commonly isolated bacteria as CoNS (239/994, 24.04%) followed by *Klebsiella* including ESBL and carbapenemase producer (236/994, 23.74%) and *E. coli* (110/994, 11.06%). Coagulase negative *S.* are the most common blood culture contaminant and very much responsible for nosocomial bloodstream infection. Since only one blood sample is usually obtained from one patient for blood culture, it is not possible to determine whether the result is due to skin contamination or really true bacteremia. But recovery of CoNS may be due to other factors like use of prolonged intravascular device, prolonged stay in hospital, or underlying comorbidities. So, our findings are consistent with many surveillance studies.[9,18,21,25] Warren et al[26] detected CoNS as a significant cause of bloodstream infection. In a study done by Jombo et al,[24] commonest blood culture isolates were *Staphylococcus aureus* (22.7%) followed by *Klebsiella* species (20.2%) and third being *E. coli* (15.3%). Similar result was obtained in the study in Yobe state, Nigeria, and also documented in Slovakia where common bacterial isolates were *E. coli*, *Staphylococcus*, and *Klebsiella* species.[27,28] Again, in a study in Warsaw, Poland, *E. coli* and *Klebsiella* were the 1st and 2nd commonest, third being *Proteus*.[29] So, in any tertiary care hospital, at least two samples of blood should be taken for culture to determine whether it is true CoNS bacteremia or it is merely contamination. One should also consider that blood should be collected very aseptically.

Our study considered penicillin, amoxicillin, oxacillin, ampicillin, cefuroxime, cefotaxime, ticarcillin, ceftriaxone, ceftazedime, cefoxitin, cefepime, azithromycin, erythromycin, aztreonam, cefoperazone, nalidixic acid, fluoroquinolone group of drugs, nitrofurantoin, tetracycline, clindamycin, vancomycin, teicoplanin, and linezolid showing low sensitivity (<50%) to Gram-negative bacilli except *Salmonella* (>50% sensitive to ampicillin, cefotaxime, cefepime, azithromycin, fluoroquinolones, and chloramphenicol), acinetobacter (>50% sensitive to cefepime) and *Pseudomonas* (>50% sensitive to cefotaxime, levofloxacin). On the contrary, these Gram-negative bacilli were very much sensitive to piperacillin, cefoperazone, imipenem, meropenem, aminoglycoside group of antibiotics, tigecycline, polymyxin B, and colistin. Gram-positive isolates were sensitive to piperacillin, cefoperazone, vancomycin, teicoplanin, and linezolid. This was similar to the study done by Jombo et al,[24] who showed 0 to 50% sensitivity to penicillin, ampicillin, cloxacillin, tetracycline, cotrimoxazole, and chloramphenicol. In our study, each recovered agent showed resistance to at least four antimicrobial agents, which underscores the depth of antibiotic resistance in contemporary medical practice. This resistance of Enterobacteriaceae has been shown in different studies in Brazil, Vietnam, as well as South Africa.[30-32] This resistance is probably due to plasmid-mediated beta-lactamase production. *Staphylococcus* in our study demonstrated sensitivity of > 60% to amoxicillin, piperacillin, ceftriaxone, erythromycin, aminoglycoside group of drugs (except tobramycin), fluoroquinolone group of antibiotics, cotrimoxazole, chloramphenicol, tetracycline, tigecycline, and clindamycin and ≥ 90% sensitivity to vancomycin, teicoplanin, and linezolid. This finding was contrary to the studies done in Asia Pacific, Germany, and Nigeria.[33-35] Again in the study done by Jombo et al,[24] *S. aureus* showed > 90% sensitivity to fluoroquinolone group of antibiotics, 63 to 70% sensitivity to aminoglycoside group of antibiotics (similar to our study), but 0% sensitivity to chloramphenicol (which is contrary to our study). But studies done by Qureshi and Aziz[17] as well as by Devi et al[18] showed nearly 100% sensitivity to vancomycin and linezolid, which is nearly similar to our study (vancomycin 96.5%, linezolid 97.67%).

CONCLUSION

Our study documented 8.58% positive cultures in last 7 years. Gram negative bacterial isolates were significantly higher than Gram-positive isolates (64.19% *vs* 34.80%).

Males were significantly culture positive than females (549/994 *vs* 445/994). Most common bacterial isolates were CoNS followed by *Klebsiella* species and *E. coli*. Since CoNS is the most common contaminant of skin, to confirm true bacteremia, blood should be taken aseptically in two occasions from the same patient. Gram-negative bacterial isolates were highly sensitive to piperacillin, cefoperazone, imipenem, meropenem, aminoglycoside group of antibiotics, tigicycline, polymyxin B, and colistin. Gram-positive bacterial isolates were sensitive to piperacillin, cefoperazone, vancomycin, teicoplanin, linezolid, and clindamycin. *Salmonella typhi* was sensitive to ampicillin, cefoperazone, cefepime, azithromycin, chloramphenicol, and fluoroquinolone group of antibiotics. *Acinetobacter* showed >50% sensitivity to cefepime and *Pseudomonas* showed >50% sensitivity to cefotaxime and levofloxacin. To prevent progressive increase in antimicrobial resistance to antibiotics in different centers, a proper guideline should be set so that ultimate aim of recovery of the severely ill patients should be fulfilled.

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REVIEW ARTICLE

Dengue: A Review

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ABSTRACT

Dengue is the most common arboviral disease in the world with over 50 million people being affected all over. Caused by the virus from genus *Flaviviridae*, it can result from nonspecific viral illness. Early diagnosis, rapid identification of the complications, and fluid restoration are the cornerstone of management of this disease.

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INTRODUCTION

"Dengue is one disease entity with different clinical presentations and often with unpredictable clinical evolution and outcome." This was the fact that expert consensus groups in Latin America (Havana, Cuba, 2007), South-East Asia (Kuala Lumpur, Malaysia, 2007), and at World Health Organization (WHO) headquarters in Geneva, Switzerland, in 2008, all agreed on. Dengue infections caused by the four antigenically distinct dengue virus serotypes (DENV1, DENV2, DENV3, DENV4) of the family *Flaviviridae* are the most important and most common arbovirus diseases in humans, in terms of geographical distribution, morbidity, and mortality. Dengue infections may be asymptomatic or may lead to an undifferentiated fever (or viral syndrome), dengue fever, or dengue hemorrhagic fever (DHF).[1]

The word "dengue" is derived from the Swahili phrase Ka-dinga pepo, meaning "cramp-like seizure."

EPIDEMIOLOGY

Dengue is currently regarded as the most important arboviral disease internationally as over 50% of the world's population live in areas where they are at risk of the disease, and approximately 50% live in dengue endemic countries.[2-6] Dengue virus infection is a major cause of disease in tropical and subtropical areas, with an estimated 50 million infections occurring each year and more than 2.5 billion people being at risk of infection.[7] The virus and its vectors have now become widely distributed throughout the tropical and subtropical regions of the world, particularly over the last half century. Significant geographic expansion has been coupled with rapid increases in incident cases, epidemics, and hyperendemicity, leading to the more severe forms of dengue. Transmission of dengue is now present in every WHO region of the world and more than 125 countries are known to be dengue endemic. Estimates of the global incidence of dengue infections per year have ranged between 50 and 200 million; however, recent estimates using cartographic approaches suggest this number is closer to almost 400 million. The expansion of dengue is expected to increase due to factors such as the modern dynamics of climate change, globalization, travel, trade, socioeconomics, settlement, and also viral evolution.[8]

Dengue has been present for centuries. The first recorded symptoms compatible with dengue were noted in a Chinese medical encyclopedia in 992 AD, however, originally published by the Chin Dynasty centuries earlier (265–420 AD), prior to being formally edited.[9] The disease was referred to as "water poison" and was associated with flying insects.

The first epidemic of clinical dengue-like illness in India was recorded in Madras (now Chennai) in 1780, and the first virologically proved epidemic of dengue fever in India occurred in Calcutta (new Celiata) and the Eastern Coast in 1963–1964,[10-13] and routine outbreaks keep on occurring every year with numbers increasing during the monsoon.

VIROLOGY

The dengue virus, a member of the genus *Flavivirus* in the family *Flaviviridae*, is a single-stranded enveloped ribonucleic acid (RNA) virus, 30 nm in diameter, which can grow in a variety of mosquitoes and tissue cultures. There are four distinct but closely related serotypes (DENV1–4). They possess antigens that cross-react with other members in the same genus such as yellow fever,

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Japanese encephalitis, and West Nile viruses. There is evidence from field and laboratory studies that there are distinct strain differences between dengue viruses.

Aedes aegypti is the most efficient vector for the virus because of its domestic habits. The female bites during the day and these mosquitoes do not travel much distance from the area of origin and may result in all members of the family being affected. Once a female bites a human with the virus, it undergoes an extrinsic incubation period of about 8 to 10 days and then is able to infect the humans. Once infected, the *Aedes* mosquito can transmit the virus for about a month.[14] Transovarian transmission is possible in dengue, but it is unclear how it would affect the epidemiology of the disease.[15] Other *Aedes* mosquitoes capable of transmitting dengue include *Ae. albopictus*, *Ae. polynesiensis*, and several species of the *Ae. scutellaris* complex. These other species also transmit the dengue virus but not as effectively as *A. aegypti.*

PATHOGENESIS

When mosquitoes feed on humans, DENV is presumably injected into the bloodstream, with spillover in the epidermis and dermis, resulting in infection of immature Langerhans cells, epidermal dendritic cells (DCs).[16,17] Infected cells then migrate from the site of infection to lymph nodes, where monocytes and macrophages are recruited, which become targets of infection. Consequently, infection is amplified and virus is disseminated through the lymphatic system. Dissemination from the lymphatic system leads to invasion of other cells of the reticuloendothelial system like splenic and liver macrophages, circulating monocytes and bone marrow. Bone marrow stromal cells have also been shown to be susceptible to infection with DENV.[18]

Following infection, mononuclear cells predominantly die by apoptosis, while abortively infected or bystander DCs are stimulated to produce the bulk of mediators that are involved in inflammatory and hemostatic responses of the host. In this regard, factors that influence the amount of target cells infected, and consequently the levels of viremia, may determine the ratio of different proinflammatory and anti-inflammatory cytokines, chemokines, and other mediators, as well as the way in which the inflammatory response affects the hemostatic system.[19]

Dengue hemorrhagic fever occurs in a patient who has dengue virus infection and also, in the past, had dengue but with a different serovar. Halstead observed that the incidence of DHF and dengue shock syndrome (DSS) peaked in two populations of young children. One peak occurred in infants (at the age of 6–9 months) who were infected with a DENV serotype different from that which had infected their mothers. The key observation there was that severe disease occurred in infants for whom maternal antibodies had declined to low, subneutralizing levels. The other peak was observed in young children who had experienced an earlier, usually mild or subclinical, infection and were later infected with a different DENV serotype. These observations led to the conclusion that subsequent infection of preimmune individuals with a different DENV serotype could exacerbate rather than mitigate disease, a phenomenon that was claimed to be caused by antibodies and termed antibody-dependent enhancement (ADE) of disease.[20]

Not all cases of DHF in humans are associated with ADE or high viral loads at presentation. In some cases, when DHF/DSS is seen, the presence of viral RNA became undetectable.[21] In general, however, a high viral load and the presence of virus on the day of defervescence are important risk factors for the development of severe disease.

When deferevescence phase occurs and fever settles down in patients with dengue, they show a high level of complement activation products C3a and C5a.[22,23] Recently, it has been found that nonstructural protein NS1 has a role in complement activation causing local and systemic generation of anaphylatoxin C5a and the terminal SC5b-9 complex. The plasma levels of NS1 and SC5b-9 complexes correlated with disease severity and they were present in the pleural fluid from patients with DSS. This is a novel finding that implicates the major role of NS1 as an important trigger for complete complement activation and the role of the terminal SC5b-9 complex in the pathogenesis of plasma leakage.[24]

It is strongly believed by many scientists studying dengue pathogenesis that a high viral load and activation of high numbers of nonprotective T cells result in a "storm" of inflammatory cytokines and other mediators, leading to the increased plasma leakage characteristic of DHF/DSS. Higher plasma levels of interleukin (IL)-1β, IL-2, IL-4, IL-6, IL-7, IL-8, IL-10, IL-13, IL-18, tumor growth factor (TGF)-1β, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ have been found in patients with severe DENV infections, in particular in patients with DSS.[19]

CLINICAL FEATURES

Dengue has a wide spectrum of clinical presentations, often with unpredictable clinical evolution and outcome. While most patients recover following a self-limiting nonsevere clinical course, a small proportion progress to severe disease, mostly characterized by plasma leakage with or without hemorrhage. After the incubation period, the illness begins abruptly and, in patients with moderate to severe disease, is followed by three phases – febrile, critical, and recovery.

Earlier dengue was classified as undifferentiated fever, classical dengue, and DHF. Dengue hemorrhagic fever was further classified as having four severity grades with grade III and IV being DSS. There have been many reports of difficulties in the use of this classification, which were summarized in a systematic literature review.[25] Due to this discrepancy, the WHO has adopted a new method to classify dengue:

- Dengue without warning signs – nausea, vomiting, rash, leukopenia, positive tourniquet test, body aches and pains.
- Dengue with warning signs – abdominal pain or tenderness, clinical fluid accumulation, mucosal bleed, hepatomegaly >2 cm, thrombocytopenia, and increasing hematocrit.
- Severe dengue – severe plasma leakage leading to shock, severe bleeding, serum glutamate-oxaloacetate transaminase and serum glutamate-pyruvate transaminase in thousands causing severe hepatitis. Impaired consciousness, involvement of other organs.

Dengue starts as an acute febrile illness and usually has three phases to the disease – febrile, critical, and recovery.

Febrile Phase

Patients typically develop a high-grade fever suddenly. This acute febrile phase usually lasts 2 to 7 days and is often accompanied by facial flushing, skin erythema, generalized body ache, myalgia, arthralgia, retro-orbital eye pain, photophobia, rubeliform exanthema, and headache.[26]

Patients may have a sore throat, an injected pharynx with nausea and vomiting being a common finding. It can be difficult to distinguish dengue clinically from nondengue febrile diseases in the early febrile phase. A positive tourniquet test in this phase indicates an increased probability of dengue. Mild hemorrhagic manifestations such as petechiae and mucosal membrane bleeding (e.g., of the nose and gums) may be seen.[27,28]

Critical Phase

Not all patients go into this phase. Only the patients who have significant capillary leakage go into this phase. The onset of the warning signs of dengue, as stated above, herald the onset of critical phase. A fall in temperature is accompanied by plasma leakage which causes exudation of plasma into the third space compartments like pleura, pericardium, and peritoneum. This results in ascites, pleural and pericardial effusions, which, if massive, can lead to respiratory distress. Leakage of plasma leads to increase in hematocrit values.

More than 20% increase in hematocrit values from the baseline signifies hemoconcentration and demands a good hydration therapy. The rise in hematocrit precedes fall in blood and pulse pressure. The significant plasma leakage lasts only 1 to 2 days. Persistent vomiting and severe abdominal pain are early indications of plasma leakage and become increasingly worse as the patient progresses to the shock state. Patient mismanaged or not treated during this phase leads to shock and subsequent multiorgan failure.

Recovery Phase

After 48 hours of the critical phase, resorption of the leaked out fluid occurs. The hematocrit falls and may fall below the normal range due to dilutional effect of resorbed fluid and aggressive hydration. Appetite returns and general sense of well-being ensues in the patient. Some patients have a confluent erythematous or petechial rash with small areas of normal skin, described as "isles of white in the sea of red."[29] Many patients have generalized pruritus in the recovery phase.

If the critical phase continues and adequate hydration has not been received by the patient, then the patients may land into DSS. This usually takes place around defervescence, i.e., on days 4 to 5 of illness (of days 3–8), and is often preceded by warning signs. Tachycardia, as always, heralds the onset of shock. In the initial phase of shock, the increased systolic pressure will maintain the perfusion and with increased peripheral resistance with result in signs of delayed capillary refilling.

A child is considered to have compensated shock if the systolic pressure is maintained at the normal or slightly above normal range but the pulse pressure is ≤20 mm Hg in children (e.g., 100/85 mm Hg) or if they have signs of poor capillary perfusion (cold extremities, delayed capillary refill, or tachycardia). In adults, a pulse pressure of ≤20 mm Hg may indicate more severe shock. Worsening shock can lead to dangerously low blood pressures causing a vicious cycle of multiorgan failure and death.

The dengue virus can have some atypical manifestations. Central nervous system involvement can lead to encephalitis. Encephalopathy in DHF is an atypical manifestation and may appear in various forms, including depressed sensitivity, convulsions, neck rigidity, pyramidal signs, headache, papilledema, myoclonus, and behavioral disorders. Postinfectious sequelae are mainly amnesia, dementia, manic psychosis, Reye's syndrome, and meningoencephalitis. Neurological involvement may occur because of intracranial hemorrhage, cerebral edema, hyponatremia, cerebral anoxia, fulminant hepatic failure with portosystemic encephalopathy, renal failure, or release of toxic products. Pathophysiology of neurological involvement may include the following factors: Direct tissue lesion caused by the virus because

of its neurotropicity, capillary hemorrhage, disseminated intravascular coagulation, and metabolic disorders.[30] Guillain–Barré syndrome, transverse myelitis, and acute disseminated encephalomyelitis all have been reported to occur in dengue.[31]

Acute hepatitis occurs on the ninth day of illness and enzymes come down after 3 weeks. Acute pancreatitis and acalculous cholecystitis can be seen and the accompanying inflammatory response to them can complicate the primary illness.[30] Deranged liver functions are common in patients with dengue infection due to direct attack on liver cells or unregulated host immune response against the virus.[32] Aspartate aminotransferase levels are more than alanine transaminase levels in patients with dengue hepatitis,[32] and this has been attributed to the more muscle involvement in patients with dengue. Severe involvement has been linked with more incidences of acalculous cholecystitis, encephalitis, renal failure, and coagulopathy.[33]

Acute renal failure mostly happens due to shock and disseminated intravascular coagulation.[34] Pericardial effusion and myocarditis causing ectopic beats can occur in dengue.[35]

Dengue hemorrhagic fever can result in acute respiratory distress syndrome. Dengue virus antigen is found in alveolar lining cells of the lung. Increased permeability of the alveolar-capillary membrane results in edema in the alveoli and interstitial spaces which lead to pulmonary dysfunction.[36] Disseminated intravascular coagulation can occur as a result of ongoing shock and thus help in perpetuating the multiorgan dysfunction or can result because of the virus itself.

DIFFERENTIAL DIAGNOSIS

- Malaria — also as endemic in India as dengue, has almost the same clinical features as dengue.
- Leptospirosis — prominent myalgias with especially calf tenderness can point to leptospirosis.
- Chikungunya — usually occurs in localized outbreaks, has similar intensity of bone pain as dengue, thus a differential in early phase.
- Viral hepatitis — liver enzymes in thousands point toward an infective etiology like hepatitis A, B, E, but severe dengue can cause hepatitis which can elevate the enzymes to such proportions.
- Influenza — pharyngeal and conjunctival injection with abdominal pain can mimic influenza.
- Rickettsial infection — rickettsial disease in India has been documented from Jammu and Kashmir, Himachal Pradesh, Uttarakhand, Rajasthan, Assam, West Bengal, Maharashtra, Kerala and Tamil Nadu, with Batra[37-40] reporting a high magnitude of scrub typhus, spotted fever, and Indian tick typhus caused by *Rickettsia conori*. Fever, headache, rash myalgias can confuse with dengue and other common infections we see daily. Weil–Felix test helps in diagnosis.
- Crimean Congo virus — Crimean Congo hemorrhagic fever (CCHF) is a zoonotic viral disease caused by tick-borne virus *Nairovirus* (family *Bunyaviridae*). The typical course of CCHF infection has four distinct phases – incubation period, prehemorrhagic phase, hemorrhagic phase, and convalescent phase. The incubation period for CCHF virus is in the range of 3 to 7 days. The mean duration is largely influenced by the route of infection, viral load, and source of infection – blood or tissue from livestock.[41]
- Severe sepsis — it can mimic DHF and DSS but a normal erythrocyte sedimentation rate can differentiate the two.[42]

DIAGNOSIS

Specific tests are widely used to detect the presence of dengue. Dengue can be detected using the following:

- Antigen — detection of ns1Ag in sera up to 3 days of fever.
- Seroconversion — detection of immunoglobulin M (IgM) titers in sera from a negative status.
- Virus isolation — using reverse transcriptase polymerase chain reaction (RT-PCR) techniques.

These tests should be done in the first five days of the illness. When a patient is infected with the dengue virus for the first time, there is persistent antigenemia for up to 2 days, after which, IgM antibodies begin to form and is detected in 50% of patients by day 3 and in 98–99% patients by day 9 of illness.[43]

Five types of serological tests have been used for the diagnosis of dengue infection: Hemagglutination-inhibition, complement fixation, neutralization test, IgM capture enzyme-linked immunosorbent assay (ELISA), and indirect IgG ELISA. The limitations of these techniques are the high cross-reactivity observed with these tests, requiring a comprehensive pool of antigens, including all four serotypes, another *Flavivirus* (yellow fever virus, Japanese encephalitis virus, or St Louis encephalitis virus), and in some areas, another virus that causes similar clinical manifestations but that is not *Flavivirus*, such as Oropouche, Mayaro, or Chikungunya viruses. Furthermore, the dengue antibodies are better detected around the 5th day of disease onset, making this technique unfeasible for rapid diagnosis.[44]

Four methods of viral isolation have been routinely used for dengue viruses: intracerebral inoculation of newborn mice, inoculation on mammalian cell cultures, intrathoracic inoculation of adult mosquitoes, and inoculation on mosquito cell cultures,[45,46] but they are done only in a handful of patients and in research centers.

According to the WHO, RT-PCR is a powerful method to be used for dengue diagnosis, but it still needs to be better standardized. Other laboratory tests may show leukopenia, thrombocytopenia, and rise in hematocrit. Liver enzymes may be deranged as well in dengue hepatitis and coagulopathy may result in deranged prothrombin time.

Thrombocytopenia of moderate degree is a usual finding associated with dengue, the reasons for which are multifactorial, which include early transient marrow suppression with damage to megakaryocytes, platelet aggregation to endothelial cells targeted by dengue fever viruses, hemophagocytosis, and finally, immune destruction of platelets, with dengue antibody complexes being found on their membrane[47-51] and falling platelets the cause of admissions and worries for the treating clinicians.

Immature platelet fraction (IPF) is a laboratory parameter which helps in diagnosing the cause of thrombocytopenia. The IPF is elevated in cases of thrombocytopenia which happens due to peripheral destruction and is depressed when the cause is bone marrow suppression. One study has found out the relation and utility of IPF in dengue. According to it, when the IPF is repeated after obtaining its basal value on day 1, it shows a rising trend, and then the rise in platelet count is imminent within 24–48 hours.[52] Thus, prophylactic transfusions of platelets can be avoided in many cases.

TREATMENT

For a disease that has such complex pathology and such diverse clinical features, the treatment remains fairly simple. Adequate hydration can well save a patient suffering from severe dengue and decrease his mortality. The WHO has formulated complete guidelines on the management of dengue including the admission and discharge criteria.[43]

The following patients, who are diagnosed with dengue, need to be admitted to the hospital:

- Any patient with warning signs of dengue (see above)
- Unable to tolerate oral feeds and dehydrated look
- End organ damage suspicion
- All pregnant patients and patients with other comorbidities like diabetes mellitus, anemia, and obesity.
- Infants and elderly.

Other patients can be effectively monitored at home under close supervision of the health care provider. Adequate hydration using coconut water, juices, oral rehydration solution can be administered to the patient. If the patient cannot tolerate the same, then admission to a hospital is necessary. Paracetamol up to 4 gm/day can be used for fever. Nonsteroidal anti-inflammatory drugs should be avoided as they may increase the risk of bleeding by functional defects of platelets and also may precipitate Reye's syndrome, especially in children. Tepid sponging can be used to decrease the temperatures as well. Daily, or in resource-limited settings, every third day, hematocrit and platelet counts need to be done to monitor the disease.

Patients who are admitted to the hospital need hydration by oral and preferably by intravenous route. The goals of fluid resuscitation include improving central and peripheral circulation, i.e., decreasing tachycardia, improving blood pressure (BP) and pulse volume, warm and pink extremities, a capillary refill time < 2 seconds, improving end-organ perfusion i.e., achieving a stable conscious level (more alert or less restless), and urine output ≥ 0.5 ml/kg/hour or decreasing metabolic acidosis.

Obtain a reference hematocrit before intravenous fluid therapy begins. Give only isotonic solutions, such as 0.9% saline, Ringer's lactate or Hartmann's solution. Start with 5–7 ml/kg/hour for 1–2 hours, then reduce to 3–5 ml/kg/hour for 2–4 hours, and then reduce to 2–3 ml/kg/hour or less according to the clinical response. Reassess the clinical status and repeat the hematocrit. If the hematocrit remains the same or rises only minimally, continue at the same rate (2–3 ml/kg/hour) for another 2–4 hours. If the vital signs are worsening and the hematocrit is rising rapidly, increase the rate to 5–10 ml/kg/hour for 1–2 hours. Reassess the clinical status, repeat the hematocrit, and review fluid infusion rates accordingly. Give the minimum intravenous fluid volume required to maintain good perfusion and urine output of about 0.5 ml/kg/hour. Intravenous fluids are usually needed for only 24–48 hours. Reduce intravenous fluids gradually when the rate of plasma leakage decreases toward the end of the critical phase. This is indicated by urine output and/or oral fluid intake improving, or the hematocrit decreasing below the baseline value in a stable patient. Patients with warning signs should be monitored by health care providers until the period of risk is over. A detailed fluid balance should be maintained. Parameters that should be monitored include vital signs and peripheral perfusion (1–4 hourly until the patient is out of the critical phase), urine output (4–6 hourly), hematocrit (before and after fluid replacement, then 6–12 hourly), blood glucose, and other organ functions (such as renal profile, liver profile, coagulation profile, as indicated).

Patients who have severe plasma leakage and severe end organ involvement require aggressive fluid management. Fluid boluses at the rate of 10–20 ml/kg may be required over 15–30 minutes in DSS. In patients with compensated shock, start intravenous fluid resuscitation with isotonic crystalloid solutions at 5–10 ml/kg/hour over 1 hour. If the patient's condition improves, intravenous fluids should be gradually reduced to 5–7 ml/kg/hour for

1–2 hours, then 3–5 ml/kg/hour for 2–4 hours, and finally 2–3 ml/kg/hour which can be maintained up to 24–48 hours. Consider reducing intravenous fluid earlier if oral fluid intake improves. The total duration of intravenous fluid therapy should not exceed 48 hours. If shock persists, and the hematocrit increases or is still high (e.g., hematocrit > 50%), repeat a second bolus of crystalloid/colloid solution at 10–20 ml/kg/hour for 1 hour. After this second bolus, if there is improvement, continue with crystalloid solution and reduce the rate to 7–10 ml/kg/hour for 1–2 hours, then continue to reduce as above.

If hematocrit decreases compared with the initial reference hematocrit (especially if the repeat hematocrit is below the baseline, e.g., <35–40% in adult females, <40–45% in adult males), and the patient still has unstable vital signs, this may indicate bleeding. Look for severe bleeding.

Cross-match fresh whole blood or fresh packed red cells and transfuse if there is severe overt bleeding. If there is no bleeding, give a bolus of 10–20 ml of colloid, repeat clinical assessment, and determine the hematocrit level. If the condition improves, give fluids accordingly to patients who do not have shock (see above).

If shock persists, change to colloid solution at the same rate with frequent boluses. Parameters to be monitored include alertness and comfort levels, vital signs, and peripheral perfusion (every 15–30 minutes until the patient is out of shock then 1–2 hourly). In general, the higher the fluid infusion rate, the more frequently the patient should be monitored and reviewed in order to avoid fluid overload while ensuring adequate volume replacement. If previously not detectable, pleural effusion and ascites should be detectable after fluid boluses. Monitor their effects on breathing. A decrease in hematocrit together with stable hemodynamic status and adequate urine output indicates hemodilution and/or reabsorption of extravasated fluids. In this case, intravenous fluids must be discontinued immediately to avoid pulmonary edema.

Recognizing when to decrease or stop intravenous fluids as part of the treatment of severe dengue is crucial to prevent fluid overload. When any of the following signs are present, intravenous fluids should be reduced or discontinued:

- Signs of cessation of plasma leakage
- Stable BP, pulse, and peripheral perfusion
- Hematocrit decreases in the presence of a good pulse volume
- Apyrexia (without the use of antipyretics) for more than 24–48 hours
- Resolving bowel/abdominal symptoms
- Improving urine output.[43]

Studies have shown several sulfated polysaccharides extracted from seaweeds have been studied and high antiviral activity against dengue virus has been observed.[53] In modern medicine, ribavirin, glycyrrhizin, and 6-azauridine have been reported to have cytostatic and inhibitory effects on the dengue virus.[54]

An adenosine analog is another promising drug currently being studied. The chemical "NITD008" is the best example.[55] Currently, the most advanced targets are the NS3/NS2B protease and NS5 RNA-dependent RNA polymerase, which have undergone high throughput screening and lead compound optimization. New targets including E, NS3 helicase, and NS5 methyltransferase are being explored.[56]

Dysregulation of the immune system with its hyperactive state has been a part of the pathophysiology of dengue, and some investigators have sought for the use of intravenous Ig for the management of severe dengue, but the results are not very productive.[57]

Although corticosteroids are not mentioned in the WHO guidelines on the management of dengue, clinicians use corticosteroids empirically based on the presumed immunological basis of the complications of dengue. The evidence base for the benefit or lack of benefit of corticosteroids in dengue is limited; previous studies have been small, with methodological flaws, less stringent randomization, and unclear allocation concealment, and were performed a long time ago. Studies so far have only been in patients with shock syndrome, and the possible effects of corticosteroids on thrombocytopenia and bleeding as well as other complications of dengue are unknown. All previous studies have been in children; the effect of corticosteroid treatment in adults with dengue infection has not been evaluated.[58]

A primary immunological mechanism that confers protection from dengue illness is virus neutralization through antibodies, and all current dengue vaccine candidates aim to elicit high levels of neutralizing antibody. The increasing cocirculation of the four dengue virus types means that a vaccine is needed that protects against all four of them; hence, the vaccine needs to be tetravalent. Moreover, the induction of protective, neutralizing antibody responses against all four serotypes of dengue virus simultaneously should avoid the theoretical concern of vaccine-induced ADE in vaccine recipients. Dengue vaccines in development are of four types: Live attenuated viruses, chimeric live attenuated viruses, inactivated or subunit vaccines, and nucleic acid-based vaccines.[1]

One is a chimeric tetravalent vaccine in which the structural genes (prM and E) of each of the four dengue viruses were inserted individually to replace those of yellow fever virus in the backbone of the yellow fever 17D vaccine. Thus, the nonstructural genes of yellow fever are provided to allow replication of the chimeric

virus, and attenuation is imparted by the yellow fever portion of the chimera. Monovalent vaccines, as well as tetravalent mixtures of all four viruses, have been given to human volunteers of varying aged in phase 1 and 2 trials in both nonendemic and endemic regions. At least two doses were required to achieve high rates of tetravalent neutralizing antibodies, and somewhat higher seroconversion rates were observed in subjects with preexisting immunity to yellow fever.[59]

CONCLUSION

Dengue is the most common arboviral disease in the world and is approaching pandemic proportions. Currently, early diagnosis and correct treatment offers the only hope for curing the disease. Environmental measures would also help in curbing the numbers by decreasing the host.

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REVIEW ARTICLE

Pancreatic Lipase Inhibitor from Food Plant: Potential Molecule for Development of Safe Anti-obesity Drug

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ABSTRACT

Obesity is a global health concern, widely recognized as the largest and fastest growing public health problem in the developed and developing countries associated with high morbidity and mortality. It is a multifactorial disease resulting in significant impairment of health. The strategies used for the treatment of obesity generally comprise of prescription of drugs and surgery. Number of basic mechanisms has been considered for obesity management but these entail serious complexities. In recent year's pancreatic lipase, a principal lipolytic enzyme secreted by the pancreas has gained importance as -obesity target. As the PL acts in the duodenum it has least involvement with the blood or brain, avoiding a lot of drug related side effects. Although PL has been considered as good target for obesity management, the drug discovery and development in this section is not abundantly explored. Numerous natural molecules have been established for pancreatic lipase inhibitory activity but only orlistat (tetrahydrolipstatin), a saturated derivative of lipstatin designed to inhibit the action of gastrointestinal lipase approved by Food and Drug Administration (FDA) for long-term usage. However, it has severe side effects. Therefore, the possible treatment of obesity using natural products is an extensive field to be explored. Several plant derived molecules including medicinal plants have been reported for their pancreatic lipase inhibitory activity. In particular pancreatic lipase inhibitor from food plants can be considered as a good source for the discovery of a safe anti-obesity agent due to possible active principle as edible component. Present review mainly focuses on the pancreatic lipase inhibitor from food plants and its potential in the development of safe anti-obesity drug.

Keywords: Obesity, Pancreatic lipase inhibitor (PL inhibitor), Plant derived.

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INTRODUCTION

Obesity is generally caused by an imbalance between energy intake and expenditure which can be most often defined as a body mass index (≥30 kg/m^2).[1] Obesity is still an exciting crossroad in reference to pharmacological management. Number of synthetic drugs came into market but could not make high impact on obesity management. Now the use of natural molecule is gaining renewed interest as potential source of new anti-obesity drugs.[2] Natural products extracted from traditional plant and microbial sources have always displayed an opportunity for the development of new therapeutic agents.[3] Lipids in diet serve as the major source of undesirable calories; the inhibition of fat digestion is a good approach for reducing fat absorption.[1] Many researchers are involved in the molecular regulation of triglyceride synthesis and in pharmaceutical approaches to reduce the fat absorption and storage for the discovery of new anti-obesity agents. Natural products provide an ample scope for the discovery of pancreatic lipase inhibitors that can be developed into anti-obesity clinical products.[3] Currently, natural products for the safe management of obesity is largely unexplored.[4] Therefore; search of new, effective and safe anti-obesity phytochemical particularly from food stuff would provide an excellent opportunity in obesity management.

Obesity and Related Complications

Obesity is a metabolic disorder caused because of the imbalance between energy intake and expenditure in which excess body fat has accumulated to the extent that it may cause adverse effect on health, leading to reduced life expectancy and/or increased health related problems. People are classified as different class of obese on the basis of their body mass index.[5] In the late 1930's the medical profession made a change in opinion on the desirability of surplus "fats" and accepted it as a health problem. This field was relatively unexplored till leptin was discovered. Leptin plays an important role in regulating energy absorption and energy expense, including appetite and metabolism. It circulates at levels proportional to body fat. It regulates the amount of food taken and energy spends by acting on receptors in the mediobasal hypothalamus.[6] Therefore, overweight and obesity can be described as an abnormal fat accumulation that display or alarm

Table 1: Basic mechanisms used for anti-obesity medication strategy

Mechanism of action
Stimulating thermogenesis
Lowering lipogenesis
Enhancing lipolysis
Suppressing appetite
Decreasing the absorption of lipids

risks to health.[7] Obesity is a multifarious disorder of heterogeneous group of conditions with multiple causes and effects. It has serious effects linked to it including coronary heart disease, high blood pressure, diabetes-2 and stroke. Obesity is also linked to higher rates of certain types of cancer, i.e., colon, rectum or prostate cancer in men and gallbladder, uterus, cervix, or ovarian cancer in women. Other obesity linkages include high cholesterol, depression, gastroesophageal heartburn, infertility, etc.

Medication and their Adverse Effects

Drug treatment of obesity is generally focused at reducing energy/food intake either by an action mainly on the gastrointestinal system or via an action through the central nervous system control of appetite and feeding (Table 1). In certain situations, there may be a necessity of prescription weight loss medication. Lot of side effects may occur due to these medicines, such as allergic reactions; respiratory, gastrointestinal, psychological, musculoskeletal and cardiovascular side effects; nervous system related side effects. Obesity is a multifarious disorder of heterogeneous group of conditions with multiple causes.

A number of drugs have been used for the treatment of obesity; though most of them have been discontinued from the market because of their adverse effects. In fact, amphetamine, rimonabant and sibutramine licenses have been withdrawn due to an increased risk of psychiatric disorders and non-fatal myocardial infarction or stroke. At present for the treatment of obesity orlistat is the only available choice.[8] Hopefully, better anti-obesity drugs will be developed with lesser side effects in future.

The anti-obesity drugs currently approved by Food and Drug Administration (FDA) for treatment against obesity exhibit a series of side effects and need additional supplements to be taken along with the drug. Elevated side effects of marketed anti-obesity drug is now major concern in obesity management (Table 2).

Table 2: Some commonly used anti-obesity medications[8]

Drug	*Adverse effects*
Phentermine#	Insomnia, tremor, ↑blood pressure and pulse rate, headache, palpitation, constipation
Diethylpropion#	As above
Topiramatea#	Paraesthesia, dizziness, altered taste, fatigue, memory impairment, somnolence, anorexia, and abdominal pain
Zonisamide#	↑Nervousness, sweating, tremors, gastrointestinal adverse effects, hypersomnia, fatigue, and insomnia
Orlistat*	Abdominal pain, bloating, flatulence, oily stools, diarrhea, ↓absorption of fat soluble vitamins

#Medications for short-term weight management or selected medications used off-label to promote weight loss; *Medication for long-term weight management;↑Increasing;↓Decreasing

Pancreatic Lipase Inhibition in Obesity Management

Among all the targets used for the treatment of obesity, altering metabolism of lipids by inhibition of dietary fat absorption using pancreatic lipase is an interesting and comparatively safe approach toward the development of an anti-obesity drug. Pancreatic lipase is a principal lipolytic enzyme secreted by the pancreas and plays a pivotal role in the digestion of fats (Fig. 1). As the pancreatic lipase acts in the duodenum and it has least involvement with the blood or brain avoiding a lot of drug related side effects and development of other complications.

In view of search for a better and comparatively safer drug target for pancreatic lipase inhibition, large number of synthetic as well as natural molecules/extracts has been investigated for pancreatic lipase inhibitory activity. However orlistat (also known as tetrahydrolipstatin), designed and developed as anti-obesity drug is the only widely available and approved anti-obesity drug for long term use a saturated derivative of lipstatin inhibits the action of gastrointestinal lipase and thus reduces absorption of dietary fat. However, it displays severe side effects with various complications which are now a major concern of its long term use.[1] It is the only weight loss medication of its kind that has been approved by the FDA. It basically blocks the digestion and absorption of fat in stomach and intestines. The fat that remains unabsorbed is excreted in the stool.

Side effects associated with orlistat include allergic reactions like hives, difficulty in breathing and swelling of face, throat, tongue, etc. Oily and frequent bowel movements, bowel urgency and gas stomach pain, nausea, vomiting, diarrhea, rectal pain are main side effects of orlistat. Respiratory side effects have included

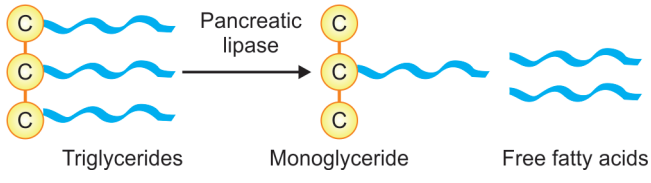

Fig. 1: Schematic representation of pancreatic lipase action

influenza, upper respiratory infections of ear, nose, throat and lower respiratory infection symptoms. Musculoskeletal side effects have noted back pain, pain in the lower extremities, arthritis, myalgia, joint disorder and tendonitis. Headache and dizziness are major nervous system side effects. Psychiatric side effects have included psychiatric anxiety and depression. This is the prime reason for researcher as they are looking for new plant derived pancreatic lipase inhibitor especially from food plant for development of safe anti-obesity drug. Natural products serve as tremendous source for pancreatic lipase inhibitors.

Plant Derived Pancreatic Lipase Inhibitor

Search of potent lipase inhibitors from plant extracts is among the various strategies employed for the discovery of anti-obesity drugs.[7] The formative variance of natural products combined with the fact that they were elaborated within the living systems provides a more sustainable choice to completely synthetic molecules.[9] Potential of natural products for the management of obesity is still broadly unexplored.[4] Natural products provide an ample scope for the discovery of pancreatic lipase inhibitors that can possibly be developed into clinical products. Hence, the focus is also on plant derived pancreatic lipase inhibitor as a potential molecule for preparing for an anti-obesity drug. Large number of plant derived components including various types extracts, phytochemicals, processed plant have been reported for inhibition of pancreatic lipase inhibitory activity. Long list of plants extracts have been investigated for lipase inhibitory activity and good number of plant extracts have been reported for lipase inhibitory activity.[10]

Inhibitory effect of some plant extracts on pancreatic lipase was presented by Gholamhoseinian and co-worker, 2010 where they have shown the percent (%) inhibition of pancreatic lipase.[10] According to the data given in a research paper percent inhibition of various plants against pancreatic lipase are as follows, *Quercus infectoria*, galls (85.0%), *Eucalyptus alba*, leaves (64.0%), *Rosa damascena*, floret (57.0%) (Fig. 2A), *Levisticum officinale,* roots (55.0%), *Urtica urens*, aerial parts (44.7%), *Alhagi camelorum*, aerial parts (44.5%), *Otostegia persica*, aerial parts (44.0%), *Rheum ribes*, rhizomes (43.0%), *Pistacia vera*, fruit hull (42.0%), *Myrtus communis*, leaves (40.0%) (Fig. 2B), *Cinnamomum Zeylanicum*, derm (39.0%), *Ficus caria*, leaves (34.2%), *Nigella sativa*, seeds (31.4%), *Pimplinella anisum*, seeds (31.0%), *Trigonella foenum-graecum*, seeds (30.0%), *Bunium persicum*, seeds (28.0%), *Carthamus oxyacantha*, aerial parts (28.0%), *Arctium lappa*, roots (26.8%), *Zingiber officinale,* rhizomes (23.4%), *Convolvulux pilosellaefolius*, aerial parts (23.3%), *Origanum majorana,* plant (23.0%), *Rubia tinctorum*, roots (23.0%), *Camellia sinensis*, leaves (22.0%), *Peucedanum aucheri*, roots (22.0%), *Outreya carduiformis*, aerial parts (21.3%), *Cordial mixa*, fruits (21.0%), *Ocimum basilicum*, seeds (21.0%) inhibition, *Olea europaea*, leaves (21.0%), *Punica granatum*, fruits hull (21.0%) , *Laurus nobilis*, leaves (20.5%), *Ducrosia assadii*, aerial parts (20.0%), *Ferula oopoda*, aerial parts (20.0%), *Teucrium scordium*, aerial parts (20.0%).*Quercus infectoria* showed the highest percent inhibition while *Ferula oopoda* and *Teucrium scordium* showed lowest percent inhibition. A plant benzoquinone embelin (2,5-dihydroxy-3-undecyl-1,4-benzoquinone) obtained from the dried fruit of *Embelia ribes* has been investigated for pancreatic lipase inhibitory activity.[11] In addition, dried berries are also

Figs 2A and B: (A) *Rosa damascene* (floret), and (B) *Myrtus communis* (leaves) with pancreatic lipase inhibitory activity
Source: Wikipedia: https://en.wikipedia.org/wiki/Rosa_%C3%97_damascena, https://en.wikipedia.org/wiki/Myrtus#/media/File:Gardenology.org-IMG_2781_rbgs11jan.jpg Date: 26/04/2016; time: 12:10 hours

reported to inhibit enzymes, such as pancreatic lipase. In view of search for pancreatic lipase inhibitors, the methanol extract of *Dioscorea nipponica* makino powder was also evaluated which showed potent inhibitory activity against porcine pancreatic lipase with an IC_{50} value of 5 to 10 µg/mL, with 4-methylumbelliferyl oleate as a substrate.[12] *D. nipponica* active components dioscin and its aglycone, diosgenin, prevented the increase of blood triglyceride level when administered orally with corn oil to mice, suggesting it appeared to have a potent inhibitor against fat absorption.

Food Plant Derived Pancreatic Lipase Inhibitor

Phytochemicals screening for anti-obesity activity particularly from edible plant would provide an excellent new strategy for addressing the issues of obesity and its complications.[3] Food plant derived lipase inhibitory molecule may be of therapeutic interest with respect to the treatment of obesity. Extracts from various selected food plants have been screened for potential lipase inhibitory activity.[13] Food plants, such as cabbage (Fig. 3A), garden pea, parsley, celery and nettle which are used in food preparations have been explored to study pancreatic lipase inhibitory activity.[14] Extracts from Bearberry, pear prepared from fruit plants have also been reported as pancreatic lipase inhibitors. Recently lipase inhibitory activity of some food stuff extracts, such as apple, yerba mate (Fig. 4A), grapevine, soybean, oolong tea (Fig. 4B), ginseng and peanut, cinnamon (Fig. 5A) have been reported.[15-16]

Figs 3A and B: (A) *Brassica oleracea* var. Capitata-Cabbage (bulb), and (B) *Magnifera indica* (stem, bark, leaves) with pancreatic lipase inhibitory activity

Figs 4A and B: (A) Yerba mate plant (leaves), and (B) Oolong tea (leaves)
Source: https://commons.wikimedia.org/wiki/File:Yerba_mate_young_plant.JPG, https://commons.wikimedia.org/wiki/File:Csinensis.jpg Date: 27/04/2016; time: 11:10 Hours

Some of the phytochemicals identified are polyphenols and saponins which inhibit pancreatic lipase activity, which could be applied in the management of the obesity epidemic. Lipase inhibitors of plant origin generally include various phytochemicals, proteins and others.

Carpesterol from ripen fruits of *Solanum stramonifolium* has been identified and investigated for pancreatic lipase inhibitory activity.[17] Carpesterol showed moderate lipase inhibitory activity with IC_{50} value of 56 µg/mL. Water extract of *Juglans mandshurica* fruit also showed strong pancreatic lipase inhibitory activity *in vitro*.[18] The extract also inhibited the normal elevation in the level of plasma triacylglycerol in rats 2 to 4 hours after oral administration of lipid emulsion. Fourteen compounds have been isolated from *J. mandshurica* fruit were subjected for their pancreatic lipase inhibitory activity. One of which showed the strongest pancreatic lipase inhibitory activity. An investigation carried out on the inhibitory effect of *Cyclocarya paliurus* extract on postprandial hyperlipemia in mice showed single dose of *C. paliurus* extract with 5 mL/kg of lard and olive oil suppressed the plasma triacylgycerol (TG) levels and prevented its rise. *C. paliurus* extract showed pancreatic lipase inhibitory activity with an IC_{50} of 9.1 µg/mL *in vitro*.[19] A study on triterpenoid saponins isolated from the fruits of *Acanthopanax senticosus* showed pancreatic lipase inhibitory activity.[20] Among the isolated compounds, silphioside F, copteroside B, hederagenin 3-*O*-b-D-glucuronopyranoside 6-*O*-methyl ester and gypsogenin 3-*O*-b-D-glucuronopyranoside showed inhibitory activity toward pancreatic lipase. Effects of ethanol extract of *Mangifera indica* L. (stem bark and leaves, Fig. 3B) on lipases (pancreatic lipase, lipoprotein lipase and hormone-sensitive lipase) as well as for the inhibition of lipolysis of 3T3-L1 adipocytes were carried.[21] Extract of stem bark and leaves of *Mangifera indica* L. inhibited pancreatic lipase and lipoprotein lipase. Methanolic extract from the leaves of *Salvia officinalis* L. was also investigated for lipase inhibition showed considerable amount of inhibitory effect on serum triglyceride elevation in olive oil loaded mice (500 and 1000 mg/kg, orally) and pancreatic lipase inhibitory activity with IC_{50}: 94 mg/mL.[22] In an interesting experimentation pancreatic lipase inhibitory activity of the rhizome of *Alpinia officinarum* (AO) and its anti-hyperlipidemic activity were investigated and measured.[23] The ethyl acetate fraction exhibited the most potent inhibition. 3-methylethergalangin was isolated from that fraction as an inhibitor of pancreatic lipase with an IC_{50} value of 1.3 mg/mL. The results suggested pancreatic lipase inhibition was responsible for the hypolipidemic activity of AO and 3-methylethergalangin. Tannin-rich extract obtained from the *Araucaria angustifolia* seed coat is also reported as an effective pancreatic lipase inhibitor.[24] Inhibition was of the parabolic non-competitive type. This interesting result was most probably due to the indirect inhibition of triglyceride absorption by inhibition of pancreatic lipase.

In past some beverage plants have also been investigated for pancreatic lipase inhibitory activity. Anti-obesity effects of oolong tea in high-fat diet-treated mice were also investigated by some research group.[15] Interestingly, they found the pancreatic lipase inhibitory activity actions of substance present in oolong tea. The results also suggested that oolong tea may be an effective crude drug for the treatment of obesity and fat liver caused by a high-fat diet. Methanolic extract of *Ilex paraguariensis* leaves was also demonstrated for porcine pancreatic lipase inhibitory activity.[25] From the methanolic extract, three new triterpene oligoglycosides, mateglycosides A, B, and C, were isolated together with eighteen known compounds. Several constituents showed inhibitory activities on pancreatic lipase. In an investigation carried out on *Cassia auriculata* a traditional medicine used for the treatment of diseases, such as hyperlipidemia, diabetes and some other disease conditions.[26] The crude extract of *cassia auriculata* exhibited pancreatic lipase inhibitory activity with an IC_{50} of 6.0 ± 1.0 µg/mL suggesting that anti-hyperlipidemic effect of the extract might be responsible for the anti-lipase activity.

Anti-obesity effect of *Platycodi radix*, aqueous extract is also investigated and interestingly it was observed that *P. radix* inhibited intestinal absorption of dietary fat by inhibiting pancreatic lipase activity.[27] Toward the search of anti-obesity mechanism of *P. radix*, experiment was performed on activity guided isolation to find active components. The entire saponin fraction of *P. radix* appeared to have a potent pancreatic lipase inhibitory activity during hydrolysis of triolein emulsified with phosphatidycholine *in vitro.* Lee et al, 2005 have demonstrated the lipid-lowering potential of aqueous extract of *Gardenia jasminoides* Ellis (GF) fruits, showed inhibition of pancreatic lipase.[28] The two components isolated from *G. jasminoides* showed an IC_{50} value of 2.1 mg/mL for crocetin and 2.6 mg/mL for crocin on triolein substrate. These compounds efficiently inhibited the increase of serum Triglyceride level, LDL cholesterol levels in hyperlipidemic mice. The results showed that the lipid lowering activity of GF and crocin was due to the inhibition of pancreatic lipase and crocin, while the metabolite crocetin, improved hyperlipidemia. Flavonoids isolated from the leaves of *Nelumbo nucifera* leaf (NLF) were examined for its *in vitro* inhibitory potential against lipase. Experiments revealed that NLF displayed high pancreatic lipase inhibitory activity with IC_{50} value of 0.38 ± 0.022 mg/mL.[29] The results suggested that NLFs could be thought of as a possible treatment

option for hyperglycemia, hyperlipidemia and obesity. In an investigation *Salacia reticulata*, a plant found in indian forests, had been examined for its anti-obesity effects.[30] Boiled extract from the roots of *S. reticulata* were used for *in vitro* study on rats. *Salacia reticulata* hot water (SRHW) soluble extract seemed to suppress the body weight with oral administration and also showed pancreatic lipase inhibitory activity thereby ceasing that polyphenolic compound of SRHW might be responsible for anti-obesity effects in rats due to the inhibition of fat metabolizing enzymes. According to a study three triterpenoid saponins, gypsosaponins were isolated from the roots of *Gypsophila oldhamiana*.[31] These showed pancreatic lipase inhibitory activity. A study on the inhibitory activities of *Taraxacum officinale extract* against pancreatic lipase *in vitro* and *in vivo* was also evaluated to determine its potential use as a natural agent for the management of obesity.[32] *Taraxacum officinale* extract were measured using 4-MU oleate as a substrate at different concentrations. *Taraxacum officinale* extract showed good inhibitory activities against pancreatic lipase. However researchers stated that furthermore studies are needed to discover the active components involved in pancreatic lipase inhibition and evaluate the effects of continuous usage of *T. Officinale* as an anti-obesity agent. A study carried out to evaluate the pancreatic lipase inhibitory activity of the extract of *Actinidia arguta* root triterpenes.[33] Coumaroyl triterpene, 3-*O*-*trans*-*p*-coumaroyl actinidic acid, ursolic acid, 23-hydroxyursolic acid, corosolic acid, asiatic acid, and betulinic acid assesses *in vitro* showed that coumaroyl triterpene had highest pancreatic lipase inhibitory activity with an IC_{50} of 14.95 μm. Another research investigation examined the protein lipase inhibitory activity and lipolytic activity of peanut (*Arachis hypogaea* L.) shell extracts (PSE) extracted in 95% ethanol, on 3T3-L1 adipocytes.[34] *In vivo* experiments on Wistar rats showed that PSE inhibited a number of lipases, including protein lipase, lipoprotein lipase, and possibly, hormone sensitive lipase and also showed increased fecal lipid excretion with respect to the control group. Experiments showed reduced triacylglycerol content in the liver, serum glucose, and insulin. Peanut (*Arachis hypogaea* L.) shell extracts may be useful as a treatment to reduce the dietary fat absorption. The observed reduction in intracellular lipolytic activity of cultured 3T3-L1 adipocytes may reduce the levels of circulating free fatty acids. The PSE actions partly *contributed* to the inhibition of fat absorption in the digestive tract and reduced the adipocyte lipolysis. The inhibitory effects of apple polyphenol extract (AP) *in vitro* and *in vivo* was also investigated in mice and humans and it revealed that AP and procyanidin considerably inhibited *in vitro* pancreatic lipase activity.[35] But polyphenols, other than procyanidin in AP (i.e., catechins, chalcones, and phenol carboxylic acids) showed weak pancreatic lipase inhibitory activities. These results concluded that oligomeric procyanidins in AP inhibited triglyceride absorption by inhibiting pancreatic lipase activity in mice and humans. In an *in vitro* study, the inhibitory activity of acacia polyphenol on lipase was measured.[36] In addition, the effects of AP on absorption of orally administered olive oil, glucose, maltose, sucrose, and starch solution in mice. They found that AP concentration-dependently inhibited the activity of lipase with an IC_{50} of 0.95 mg mL^{-1}. In ICR mice, olive oil was administered orally immediately after oral administration of AP solution and plasma triglyceride concentration was measured. It was found that AP significantly inhibited the rise in plasma triglyceride concentration after olive oil loading. Results suggested that AP inhibits lipase and glucosidase which leads to a reduction in the intestinal absorption of lipids.

Several proteins from plant origin have been reported for pancreatic lipase inhibitory activity. Discovering, designing, and formulating a protein and peptide drug for delivery though gastrointestinal tract are major concern and it requires a multitude of strategies. Besides strategic difficulties for making protein as anti-obesity agent various proteins are identified as pancreatic lipase inhibitor, such as those from soybean and from wheat bran. Other proteins that strongly inhibit hydrolysis of triglycerides are the basic protein protamine[37] and ε-polylysine.[38] Protein isolated from the seeds of Moringa have also been reported for pancreatic lipase inhibitory activity.[39]

Researchers have also evaluated the potential inhibitory activities of spices. According to research carried out by Etoundi et al,[41] on 19 commonly used Cameroonian spices for their polyphenol content, as well as their *in vitro* anti-lipase activities indicated that the *Xylopia aethiopica* (92.25%) and *Scorodophloeus zenkeri* (with husk) (56.39%) were most effective in inhibiting the activity of pancreatic lipase.[40] A research study carried out to check the pancreatic lipase inhibitory activity of *Illicium verum* (Fig. 5B) showed an inhibition of 22.7%.[41]

Under *in vitro* conditions ethanolic extracts of seeds of *Aframomum melegueta* presented pancreatic lipase inhibitory activities in a concentration-dependent manner.[42] Lipase inhibitory activities of 90% was observed in *A. meleguetta* at certain concentration.

CONCLUSION

As pancreatic lipase inhibition is considered as good drug target for obesity management, molecule from natural origin can be a good drug candidate for designing of safe anti-obesity drug particularly those derived from edible food plant. Voluminous scientific reports are available in

Figs 5A and B: (A) Cinnamon-*Cinnamomum verum* (bark), and (B) Star anise-*Illicium verum* (fruits and seed) with pancreatic lipase inhibitory activity

public domain on various plant including food plant and their products for its anti-obesity and pancreatic lipase inhibitory activity. Pancreatic lipase inhibitors especially from food plant can be explored for development of safe anti-obesity drug for long term use due to possible active principle as edible component.

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CASE REPORT

Dyskeratosis Congenita: A Rare Case

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ABSTRACT

Dyskeratosis congenita (DKC) is a rare genodermatosis which exhibits oral leukoplakia, nail dystrophy, and reticular skin pigmentations as its primary features. Dyskeratosis congenita has increased risk of developing constitutional anemia and malignancies and early diagnosis enables the patient to be monitored and proper interventional therapy to be instituted. Here, we present an interesting and rare case report of DKC. Very few are being reported in our country and we, as physicians, should be aware of DKC, presenting as pyrexia, and anemia.

Keywords: Dyskeretosis congenita, Leukoplakia, Pancytopenia, Telomere.

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INTRODUCTION

Dyskeratosis congenita (DKC) is a rare inherited bone marrow failure syndrome characterized by the triad of dystrophy of the nails (90%), reticular skin pigmentation (90%), and oral leukoplakia (80%). It is associated with a high risk of developing aplastic anemia, myelodysplastic syndrome, leukemia, and solid tumors. Atresia of the lacrimal ducts may occur causing continuous lacrimation. Patients have very short germ line telomeres. Hence, many of the associated symptoms like premature graying are characteristic of geriatrics and the tissues affected are those with a high cell turnover. Dyskeratosis congenita is a rare disease and usually presents to a dermatologist for skin, nail, and hair changes. However, here we present a case who presented with constitutional symptoms to us after careful general examination revealed triad of presentation of this rare disease highlighting the importance of careful head to toe examination in every patient.

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CASE REPORT

A 20-year-old male student, resident of Karjat, Maharashtra, presented with chief complaints of fever, weight loss and dyspnea on exertion since the last 6 months. Fever was moderate grade, intermittent and associated with night sweats. He had dyspnea on moderate exertion with no accompanying orthopnea. Considerable weight loss of 12 kg was present in the patient. There was no significant past, family or treatment history. No family member was dealing with history of intrauterine growth retardation, short stature, family history of abnormal toe nasils, leukoplakia, neck cancer, hypogonadism, and premature gray hair. On examination, patient was febrile and was dehydrated. His general examination revealed severe pallor, reticular hypopigmentation, and hyperpigmentation on the entire skin and dystrophic nails along with presence of multiple leukoplakias in the oral cavity. There was also presence of graying of hair. On investigations, patient had severe anemia with hemoglobin of 4.2 gm/dl, total leukocyte count of 1100 cells/mm^3 and platelet count of 10,000 c/mm^3. Rests of the laboratory parameters were normal. Patient was subjected for a bone marrow examination and genetic examination. Bone marrow examination revealed a hypoplastic bone marrow and genetic studies could not be done because of financial constraints.

Diagnosis of DKC was made with constellation of presence of skin changes, dystrophic nails, early graying of hair, and pancytopenia. Patient was managed conservatively with intravenous fluid, blood transfusions and antibiotics (Figs 1 to 4).

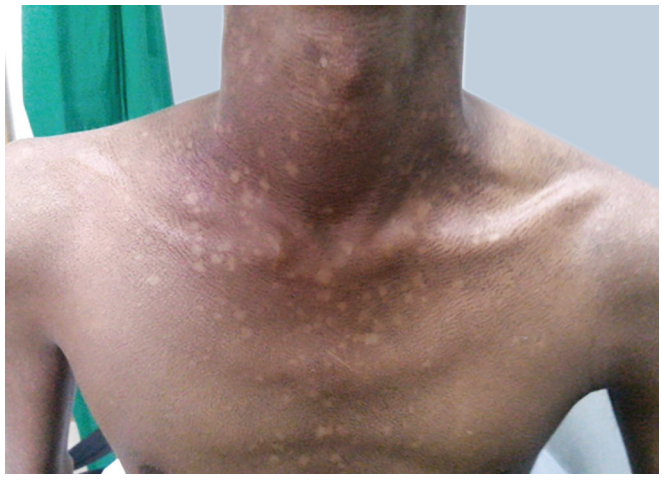

Fig. 1: Hypopigmented patches on the neck and chest region

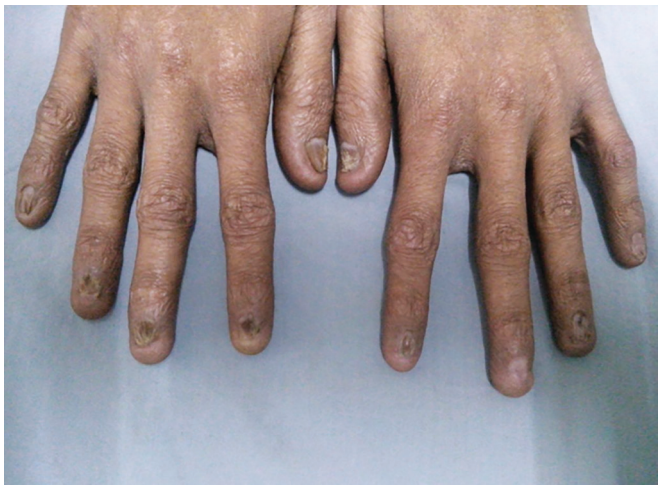

Fig. 2: Dystrophic nail

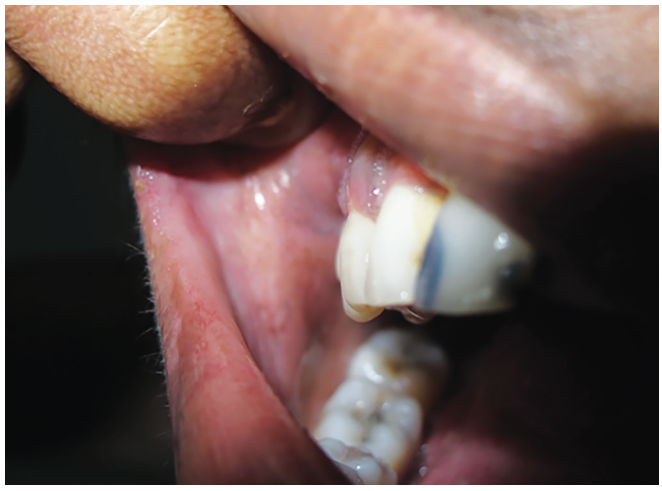

Fig. 3: Oral leukoplakia

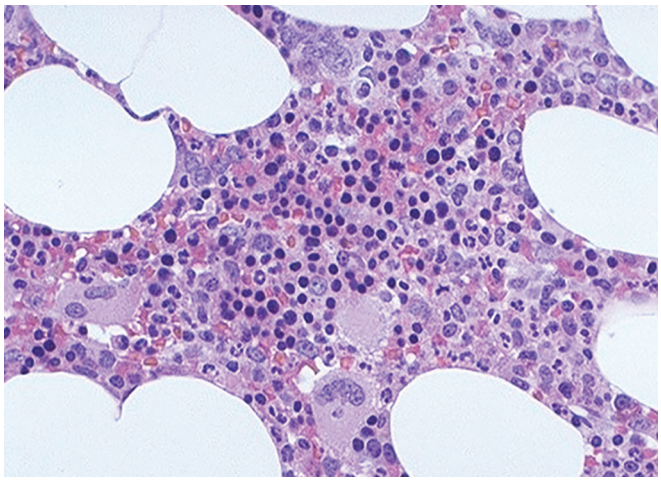

Fig. 4: Pancytopenia in peripheral blood smear

DISCUSSION

Dyskeratosis congenita, an inherited syndrome, first described by Zinsser in 1910, is characterized by the triad of reticulated skin hyperpigmentation, nail dystrophy (both occurring in 100% of cases), and white plaques (80% of cases); typically occurring in the oral cavity[1] Other features occur with lower frequencies and involve virtually every organ system.[2]

The main causes of death are bone marrow failure/immunodeficiency (~60–70%), pulmonary complications (10–15%), and malignancy (5–10%).[3] Dyskeratosis congenita is related to telomerase dysfunction; all genes associated with this syndrome (DKC1, TERC, TERT, TINF2, and NOP10) encode proteins in the telomerase complex responsible for maintaining telomeres at the ends of chromosomes. Patients with DKC have reduced telomerase activity and abnormally short tracts of telomeric DNA compared with normal controls. Telomeres are repeat structures found at the ends of chromosomes that function to stabilize chromosomes, they have critical role in preventing cellular senescence and cancer progression. The defective telomere maintenance in DKC results in chromosomal shortening and gene loss during cell replication which ultimately leads to cell apoptosis, particularly in highly proliferative tissues, such as the hematologic and dermatologic systems.[4]

Two subsets of DKC have been reported:

Hoyeraal-hreidarsson (HH) syndrome: The clinical findings are consistent with DKC, plus intrauterine growth retardation, developmental delay, microcephaly, cerebellar hypoplasia, immunodeficiency, and bone marrow failure.

Revesz syndrome: Findings similar to HH, plus a specific finding in the eye, called "exudative retinopathy".

The typical symptoms of DKC involve the skin, nails, and mucous membranes, as well as bone marrow failure. The cutaneous presentation is abnormal skin pigmentation with tan-to-gray hyper pigmented or hypo pigmented macules and patches in a mottled or reticulated pattern, which may clinically and histologically resemble graft *vs* host disease. The typical distribution involves the sun-exposed areas, including the upper trunk, neck, and face as seen in our patient.[5] Other cutaneous findings may include alopecia of the scalp, eyebrows, and eyelashes, premature graying of the hair, hyperhidrosis, hyperkeratosis of the palms and soles, and adermatoglyphia (loss of dermal ridges on fingers and toes). Nail dystrophy, the first component of the syndrome to appear, is seen in approximately 90% of patients, with fingernail involvement often preceding toe nail involvement. Progressive nail dystrophy begins with ridging and longitudinal splitting. Progressive atrophy, thinning, and distortion eventuate in small, rudimentary, or absent nails.[6] In mild cases ridging and longitudinal fissuring occur. In our patient all the toe and finger nails were dystrophic from birth itself.

Though mucosal leukoplakia commonly involves the buccal mucosa, tongue, and oropharynx, it may also be seen in areas like esophagus, urethral meatus, glans penis, lacrimal duct, conjunctiva, vagina, anus, etc. Constriction

and stenosis can occur at the later mentioned sites, with subsequent development of dysphagia, dysuria, phimosis, and epiphora. The leukoplakia may become verrucous, and ulceration may occur.[7] Leukoplakia of the buccal mucosa and hyperpigmentation of the tongue were found in our patient. Bone marrow failure is a major cause of death, with approximately 70% of deaths related to bleeding and opportunistic infections as a result of bone marrow failure. Approximately 90% have peripheral cytopenia of one or more lineages. In some cases, this is the initial presentation with a median age of onset of 10 years.[8] There was no hematological abnormality in our patient.

Individuals with DKC may also be presented with gastrointestinal system findings like hepatosplenomegaly and cirrhosis and pulmonary complications, including pulmonary fibrosis and abnormalities of pulmonary vasculature. Other symptoms, such as an increased prevalence and severity of periodontal disease, increased incidence of dental caries as in this patient, mandibular hypoplasia, osteoporosis, and scoliosis may be seen in these types of patients. Abnormalities of the CNS like low intelligence, small sella turcica and intracranial calcifications have also been reported.[3] Patients have an increased prevalence of malignant mucosal neoplasms, particularly squamous cell carcinoma of the mouth, nasopharynx, esophagus, rectum, vagina, or cervix. These often occur within sites of leukoplakia and tend to develop in the third decade of life. The prevalence of squamous cell carcinoma of the skin is also increased. Other malignancies reported include Hodgkin lymphoma, adenocarcinoma of the gastrointestinal tract, and bronchial and laryngeal carcinoma.

Dyskeratosis congenita is usually diagnosed by taking in to account the findings on physical examination and with the help of telomerase length testing and mutation analysis. The type of DKC with X-linked inheritance shows mutations in the gene called DKC1, whereas DKC with autosomal dominant inheritance may be due to mutations in other genes called TERC, TERT, and TINF2 and autosomal recessive type of DKC is characterized by abnormal genes called NOP10 (also known as NOLA3). The diagnosis of DKC in our patient was supported by the presence of characteristic triad of pigmentary and atrophic changes of the skin nail dystrophy and leukoplakia on the buccal mucosa. In addition, he had variety of minor manifestations like multiple caries teeth, gingivitis, hyperpigmentation of tongue, gastric ulcer, skeletal abnormalities, and features of premature aging as have been described in earlier reports.

Currently, there is no curative treatment for DKC. The variation in presentation makes it difficult to treat, with bone marrow failure/immunodeficiency being the main cause of premature mortality. Use of the anabolic steroid oxymetholone and hematopoietic growth factors, such as erythropoietin (epoetin alpha), granulocyte macrophage colony—stimulating factor and granulocyte colony—stimulating factor (filgrastim) can produce improvement in the hematopoietic function.[9] Although the mechanism of action of oxymetholone is not well understood, it is thought to function by promoting the growth of hematopoietic progenitors indirectly through the effect of cytokine production and by supporting hemopoietic production in times of stress. The only long-term cure for the hemopoietic abnormalities is allogeneic hematopoietic stem cell transplantation, but this is not without risk. There is still significant mortality associated with bone marrow transplants for DKC patients when compared with other bone marrow failure syndromes. One of the main reasons for this is the high level of pulmonary/vascular complications that present in these patients probably as a result of the underlying telomere defect.[10]

CONCLUSION

The advances in understanding of DKC have increased remarkably over the last 10 years but there are still huge advances to be made. The long-term survival, however, is unknown at present but the initial response is encouraging as a more effective treatment for DKC.

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CASE REPORT

Ileal Ureter for Panureteral Stricture of Tubercular Origin

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ABSTRACT

Genitourinary tuberculosis (GUTB) is the second most common extrapulmonary tuberculosis (TB) after tubercular lymphadenitis. About 8 to 15% of TB patients suffer from GUTB.[1] The most common age of GUTB presentation is the fourth decade and the commonest organ involved is kidney. We report here an unusual case of 20-year-old male patient with panureteral tuberculous stricture for which he underwent successful ileal ureter replacement.

Keywords: Genitourinary tuberculosis, Ileum, Panureteral stricture.

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INTRODUCTION

Tuberculosis (TB) has been a leading public health problem, especially in the developing countries of Asia, causing approximately 3 million new cases and 7 lakh deaths every year.[1] Genitourinary tuberculosis (GUTB), the second commonest extrapulmonary form of TB, caused by hematogenous spread of organism through blood stream. It affects males and females equally and is commonest in the fourth decade of life. The kidney is usually the primary organ affected in the urinary system, and most other parts of the urinary tract are involved due to direct extension. Insidious onset and difficulty in diagnosis may lead to delay in treatment. This may result in serious complications, such as destruction of kidney or severe involvement of the urinary bladder. Management of ureteral stricture poses both a diagnostic dilemma as well as taxes the surgical skills of the reconstructive surgeon as it is long segment, multiple and associated with fibrosis. If not properly managed, the kidney may be lost. Similarly, the clinician should be careful in declaring the prognosis of these cases as the outcome of ureteral involvement is also dependent on the extent of renal involvement.[2,3] The purpose of this case report is to discuss the investigations and management for tuberculous panureteric stricture at unusual age.

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CASE REPORT

A 20-year-old male patient was presented as a diagnosed case of left-sided panureteral stricture with left-sided percutaneous nephrostomy (PCN) *in situ* since 9 months. There was history of repeated urinary tract infection (UTI), requiring hospitalization and intensive care unit (ICU) management due to urosepsis secondary to blocked PCN. Previously, the patient was admitted to a private hospital, with history of repeated UTI, painful hematuria without clots, dysuria, and left flank pain on and off for 1.5 years. There is no past history of Koch's or Koch's contact. In the private hospital, he was evaluated with revealed microscopic hematuria and pyuria with Mantoux test positive. Complete blood count (CBC) and renal function test was normal, erythrocyte sedimentation rate (ESR) was 20. Urine culture for acid-fast bacillus was negative. Chest and abdomen X-rays were normal. On ultrasound of the abdomen, there was mild-to-moderate hydronephrosis on left side with ureteric thickening and thickened irregular bladder wall s/o infective etiology. Computed tomography and intravenous urography (CT-IVU) revealed left-sided delayed excretion with diffuse left ureteral wall thickening and luminal irregularity in the entire course with diffuse narrowing of left lower ureter and pelvi-ureteric junction (PUJ). There were multiple enlarged left renal hilar, para aortic, and small aorto caval lymph nodes (Fig. 1). So the patient was subjected for diagnostic cystoscopy and bladder biopsy which showed presence of tuberculous granuloma on histopathology.

The patient was started on four drug antitubercular treatment (ATT), and after completion of 1 month of ATT the patient was subjected to intravenous urography (IVU) examination, which showed left-sided moderate hydronephrosis with PUJ obstruction. After 2 months of ATT, the patient developed left flank pain with fever for which Ultrasonography (USG) abdomen was done, revealing progression of left hydronephrosis with internal echoes s/o pyonephrosis. Double J (DJ) stenting was attempted twice but failed so following this PCN was done on left side. On follow-up percutaneous nephrostogram was done which showed, abrupt cut-off at renal pelvis, with no visualization of PUJ or distal ureter. The patient

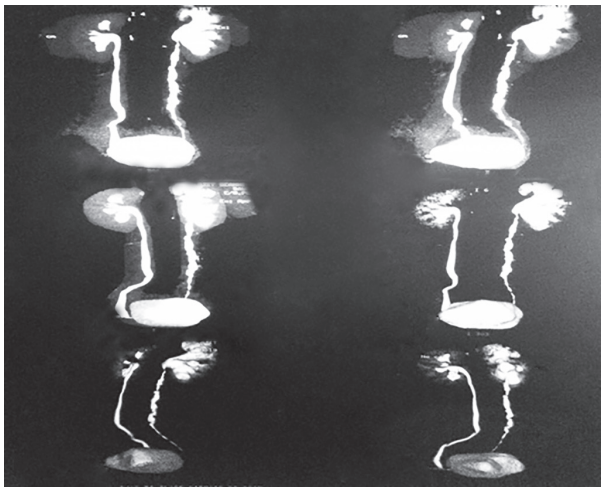

Fig. 1: Computed tomography and intravenous urography showing diffuse wall thickening and luminal irregularity in entire left ureter with moderate luminal narrowing at pelvi-ureteric junction and diffuse narrowing of lower ureter. Left ureter is showing beaded appearance s/o panureteral stricture

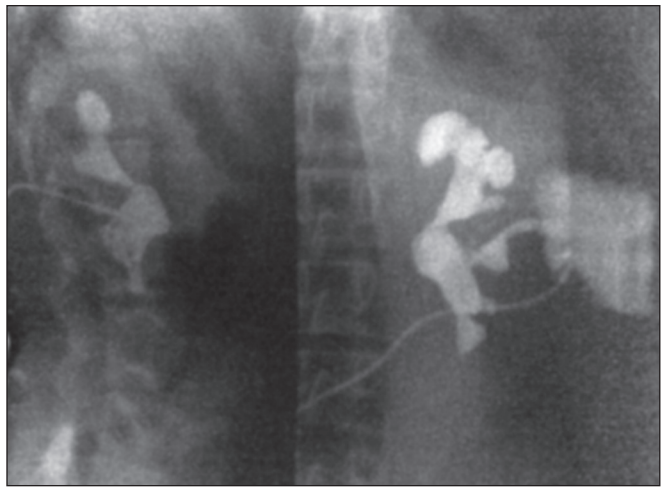

Fig. 2: Percutaneous nephrostogram complete cut off at left pelvis with no visualization of ureter

was on PCN for 1 year which was getting blocked by developing pyonephrosis and sepsis, requiring admission for IV antibiotics on and off (Fig. 2). To see the *salvagiblity* of left kidney diuretic Diethylenetriamine Pentaacetic Acid (DTPA) scan was done which showed left kidney with moderately impaired function and obstructed drainage pattern (36%) with mild impairment in glomerular filtration rate. Urodynamic study was performed for capacity, compliance, and outlet obstruction, which were normal. So ileal ureter was planned in view of salvageable kidney and panureteral stricture after confirming normal ileum on barium meal follow through.

The patient underwent left polar guillotine type of partial nephrectomy with ureterocalicostomy (ileo-ureterostomy) as even renal pelvis was fibrosed, through 11th rib bed incision using 25-cm-long ileal segment with nephrostomy and DJ stenting. Postoperative period was uneventful, and the patient was subjected to sodium bicarbonate irrigation. On 10th postoperative day, cystogram revealed no extravasation. On the 14th day, IVU revealed left-sided good renal function and excretion with no extravasation at both anastomosis sites (Fig. 3).

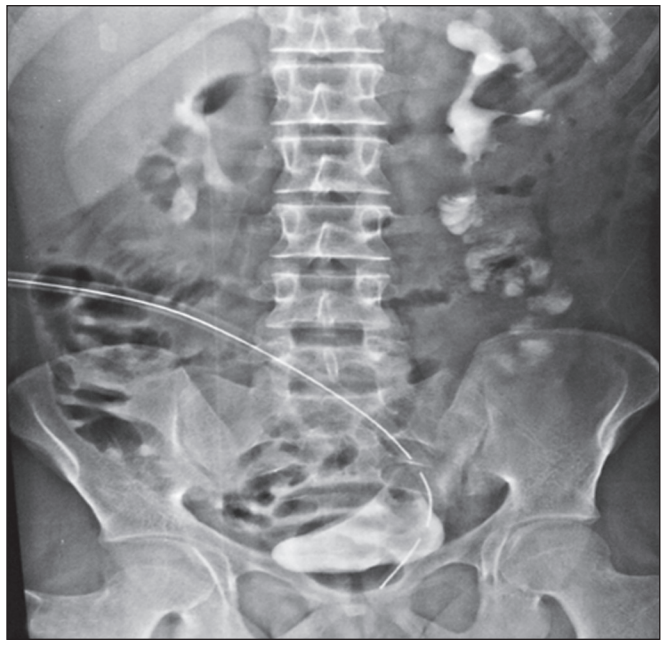

Fig. 3: Postoperative intravenous pyelogram showing good function and continuity at ileovesical and ileocecal junction

DISCUSSION

The purpose of presenting this case is that the mainstay or cornerstone in treatment of genitourinary Koch's is antituberculous drugs. However, with successful treatment with ATT, healing process results in fibrosis. Thus tuberculous healing process can lead to disastrous complications. There are ample studies which have emphasized that if there is any suspicion of involvement of ureter by TB process, before starting ATT ureter should be stented. Stenting causes passive dilatation and prevents further worsening of pathological narrowing by acting as a splint. So if the previously treating doctor would have stented the ureter before starting ATT may be this disastrous complication could have been avoided.

Commonest site of tubercular ureteric stricture is UVJ > PUJ > middle third. Length of stricture varies but commonest < 5 cm. Panureteral stricture is very rare. Ureteric stricture resulting obstructive uropathy can lead to renal function loss. Cornerstone in the treatment of GUTB is medical line of treatment by ATT. Unfortunately, as healing starts with ATT, fibrotic changes develops resulting into devastating complications like ureteric stricture. By definition, complex ureteric stricture is a long segment, extensive/bilateral, nonpassable stricture with or without

salvageable kidney and bladder. Commonest indication of ileal ureter is long ureteric stricture from secondary to TB, schistosomiasis, radiation, or traumatic ureteral loss. Review literature states that management of ureteral stricture of tubercular origin poses both a diagnostic dilemma as well as taxes the surgical skills of the reconstructive surgeon.[4,5] Literature states that ileal ureter should be performed in very selected parts if no other modality is possible. This procedure involves significant peri- and postoperative morbidity and long-term follow-up.[6]

The few contraindication for ileal ureter are impaired renal functions (creatinine > 2 mg/dl), untreated bladder outlet obstruction, incontinence, neurogenic bladder, ileal disease, and hepatic dysfunction.[7] In some patients, metabolic complications are encountered after surgery. These are: Hyperchloremic acidosis, osteomalacia, abnormal drug metabolism and altered sensorium. Other complications that can occeer, arise from removal of portions of gut from the intestinal tract like items, intestinal obstruction stricture, bowel or urinary leakage and abdominal abscess. Other reported complication are urothelial and bowel malignancies, stone formation and renal failure.[8]

CONCLUSION

Genitourinary tuberculosis remains a cause of morbidity, particularly in developing countries, where the incidence of TB is very high. Also the delay in diagnosis leads to further increase in morbidity. Early DJ stenting should be done after 4 to 6 weeks of ATT whenever structural changes in ureter is suspected to salvage the kidney and prevent renal failure. With improved multidrug therapy and reconstructive surgery satisfactory outcome is possible.

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CASE REPORT

Isolated Bladder Endometriosis: A Rare Case Report

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ABSTRACT

Endometriosis is defined as presence of functional endometrial glands and stroma beyond the normal confines of the uterus.[1,2] Overall incidence is 10 to 20% of women in reproductive age, with peak incidence between 30 and 45 years.[2] About 40% of women with infertility and 60% of those presenting with chronic pelvic pain have endometriosis.[1] About 1% of women with endometriosis have urinary tract involvement, of which 84% involve the bladder. Urinary bladder endometriosis as a part of deep infiltrating pelvic endometriosis is known, but isolated bladder involvement is extremely rare. Patients present with vague and distressing urinary symptoms mimicking recurrent cystitis, hence strong clinical suspicion with prompt recognition of this entity is important to avoid prolonged morbidity.[2] We report a case of isolated bladder endometriosis in a 28-year-old female with previous two cesarean sections. Open partial cystectomy was performed. Histopathology of the excised mass was diagnostic.

Keywords: Endometriosis, Isolated, Partial cystectomy, Urinary bladder, Urinary symptoms.

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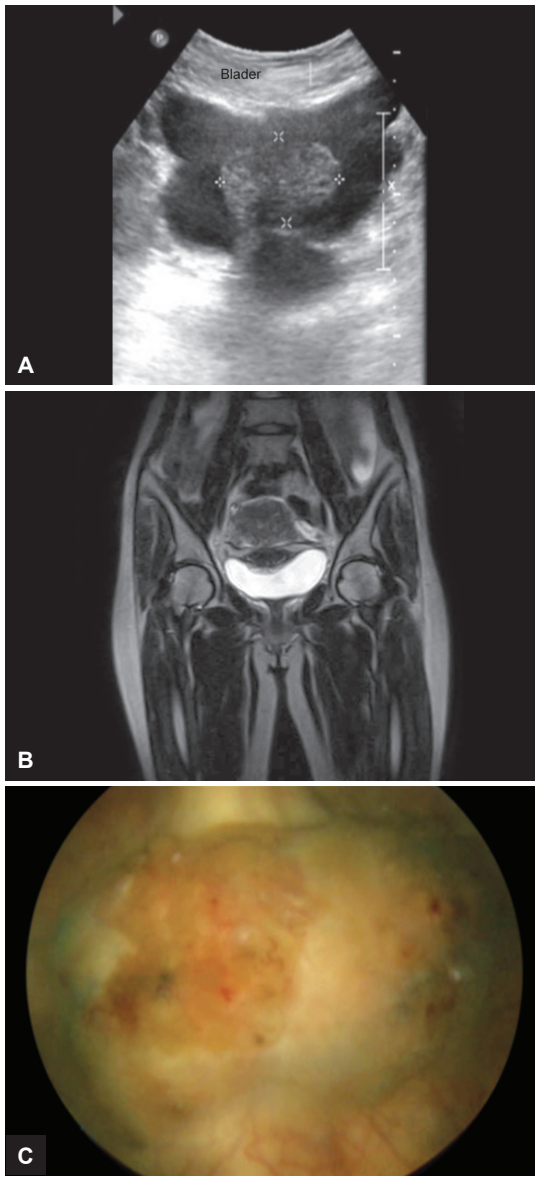
CASE REPORT

A 28-year-old, married woman presented with chief complaints of cyclical episodes of frequency, urgency, dysuria, and lower abdominal pain during premenstrual and menstrual period, for 15 days every month for last 4 years. There was no history of hematuria. Menstrual cycles were regular. The patient was married for last 10 years and had a history of three intrauterine deaths – all at full term, the 1st being normal delivery followed by two cesarean sections. The last cesarean section was performed 5 years back. Abdominal examination revealed healed scar of previous cesarean section. Pelvic examination was normal.

Ultrasonography (USG) suggested echogenic polypoidal mass projecting from posterior bladder wall (Fig. 1A). A pelvis magnetic resonance imaging (MRI) scan showed 3.7 × 3.3 × 2.7 cm heterointense lesion with smooth indentation along posterosuperior wall of urinary bladder with loss of fat planes between anterior myometrium and bladder serosa (Fig. 1B). Cystoscopic examination identified a sessile, irregular, nodular, bluish mass in the midline, at the junction of dome and posterior wall of bladder (Fig. 1C). Histopathology of cystoscopic biopsy suggested endometriosis of bladder.



Figs 1A to C: (A) Ultrasonography showing echogenic mass arising from posterior bladder wall, (B) magnetic resonance imaging showing heterointense mass involving posterior bladder wall, and (C) cystoscopy showing nodular bluish mass arising from posterior bladder wall

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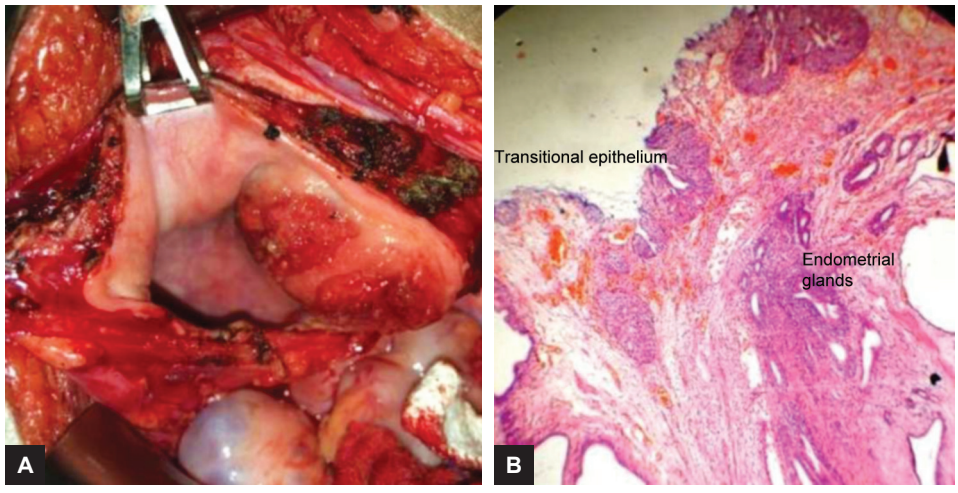

Figs 2A and B: (A) Intraoperative picture showing mass along posterior bladder wall, (B) histopathological examination showing transitional epithelium with endometrial glands

On exploration, posterior bladder wall was separated by sharp dissection from adherent anterior wall of uterus. The mass (Fig. 2A) was completely resected and bladder wall was closed in two layers. Histopathology of excised specimen revealed transitional epithelium with intact basement membrane and plenty of round tubular and cystic dilated endometrial glands (Fig. 2B) in compact cellular stroma underneath. The glands were seen infiltrating into detrusor muscles, suggestive of endometriosis of urinary bladder.

DISCUSSION

Infiltration of endometrial glands into the uterine muscle is referred to as "adenomyosis" and outside the uterus it is called "endometriosis."[2] Theories for pathogenesis of endometriosis: (1) Transplantation theory – extension of an adenomyotic nodule of the anterior uterine wall; (2) Embryonal theory — developing from Müllerian duct remnants in the vesicouterine/vesicovaginal septum; and (3) Sampson's migratory theory supported by Vercellini et al — the menstrual effluent containing viable endometrial cells can be transported to ectopic sites.[2,3] This theory has been supported by the ability of endometrial tissue to engraft itself in other areas, and this is observed in many cases of urinary tract endometriosis in patients who have undergone previous uterine surgery.[2] In our case, migratory theory with intraoperative dissemination of endometrial cells during previous caesarean section may be the etiology of vesical endometriosis. Urinary tract endometriosis predominantly affects the bladder, followed by the ureter and the kidney in the ratio of 40:5:1.

Bladder endometriosis could be primary or secondary. The primary form is spontaneous, commonly in association with deep infiltrating pelvic endometriosis (11%). The secondary manifestation results following pelvic surgery, such as cesarean section and hysterectomy.[2,3] Up to 50% of patients with bladder endometriosis have a history of previous pelvic surgery.[3] Bladder endometriosis can be intrinsic (full thickness) or extrinsic, involving serosa and peritoneal surface, generally found in the trigone, dorsal wall, or ureterovesical junction.

The symptoms of bladder endometriosis vary depending on the location and size of the lesion. About 30% patients are asymptomatic and 70% present with urinary symptoms at the time of diagnosis.[3] Lower urinary irritative voiding symptoms, such as frequency, urgency and dysuria, suprapubic pain, and hematuria are common, mimicking recurrent cystitis without bacteriuria.[3,4] Urinary symptoms are catamenial, occurring in temporal "cyclic" relationship to monthly menstruation in about 40% of the patients, while majority (60%) present with noncyclical symptoms.[4]

Ultrasonography is the initial diagnostic modality of choice in suspected bladder endometriosis. Localized bladder wall thickening can be appreciated, leading to the differential diagnosis of bladder endometriosis, subserosal anterior leiomyoma, and bladder cancer.[1] A pelvis MRI can accurately delineate the morphologic abnormalities of bladder endometriosis and also potentially identify other common sites, particularly at the uterosacral ligament. The diagnosis of vesical endometriosis is difficult, and it should be confirmed by cystoscopy with biopsy.[5] The endometrioma may show marked congestion and edema with translucent bluish nodules on cystoscopy. Bladder endometriosis can be classified as superficial (< 5 mm) or deep (> 5 mm), with depth of lesion reflecting severity of symptoms and guiding therapeutic management. Urinary bladder endometriosis may be treated surgically or medically with hormone-suppressive therapy.[5] Treatment needs to be individualized according to patient's age, severity of symptoms, extent of disease, associated pelvic disease, and parity. Hormonal treatment involves oral

contraceptives, danazol, progestin, and gonadotropin-releasing hormone agonists.[1,2] However, medical treatments usually are only palliative, and symptoms generally recur on discontinuation.[5] Surgical treatment is therefore definitive treatment for endometriosis. Type of surgical treatment depends on size and location of lesion. Small, superficial symptomatic lesions can be cauterized or ablated with bipolar or CO_2 laser. Full thickness larger lesions, as in our case, require open or laparoscopic segmental resection of bladder.

CONCLUSION

Diagnosis of bladder endometriosis is often difficult to make because of its nonspecific symptoms.[6] It requires high index of suspicion in premenopausal women complaining of catamenial bladder symptoms with negative urine cultures.[4] The management is mainly surgical and resection should be complete.[6] Partial cystectomy is the most effective treatment of choice for large full thickness lesions with high success rate and recurrence.[2] Laparoscopic and open approaches have comparable results.

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CASE REPORT

High CA 125 in a Case of Abdominal Tuberculosis mimicking Ovarian Malignancy

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ABSTRACT

A 45-year-old patient was admitted with history of abdominal pain and distension. Clinically diagnosis was pointing toward a case of right-sided ovarian mass with ascites. Computed tomography (CT) scan of the abdomen was suggestive of mucinous cystadenoma of right ovary with moderate ascites. Ascitic fluid tap was exudative in nature and negative for malignant cells. Blood investigations were within normal limits except for raised CA 125 (more than 1000 mIU/L) and raised erythrocyte sedimentation rate (ESR) (112 mm/h). Our provisional diagnosis was serous cystadenocarcinoma right ovary or pelvic tuberculosis (TB) involving right adnexa and pelvic peritoneum. Ascitic fluid findings were more in favor of pelvic TB, therefore the patient was started on antitubercular treatment (ATT) on trial basis. The patient responded considerably well to ATT.

Keywords: Abdominal tuberculosis, Ascites, CA 125.

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Conflict of interest: None

INTRODUCTION

Tubo-ovarian (TO) mass with raised CA 125 level and ascites mostly indicate an underlying ovarian malignancy. Many consider CA 125 levels more than 100 mIU/L almost synonymous with ovarian malignancy. However, CA 125 has low sensitivity and specificity as far as diagnosis of ovarian malignancy is concerned. There are many benign conditions where CA 125 can be abnormally high. One such case is reported here.

CASE REPORT

A 45-year-old patient was admitted with history of abdominal pain and distension of 5 months' duration. She did not have any significant past medical or surgical history. On clinical examination the patient was thin-built, and all vital parameters were within normal limits. Abdomen was distended and an ill-defined nontender mass was palpable in the right iliac fossa. On pelvic examination uterus was found to be bulky, and there was an approximately 6 × 5 cm size mass in right fornix. Laboratory Investigations: Hb 10.7 gm/DL, white B coo (WBC) 7800 cells/mm^3, platelets 3.80 lakh/L, ESR (erythrocyte sedimentation rate) 112 mm/h, and CA 125 more than 1000 mIU/L. Ultrasonography (USG) abdomen findings: uterus 8.1 × 4.2 × 5.2 cm anteverted, nongravid, and free fluid in pelvis. The right ovary enlarged 4.7 × 1.9 × 1.3 cm with cystic components of 2.6 × 2.2 × 2.6 cm with multiple septations. Computed tomography (CT) scan abdomen and pelvis suggested: mucinous cystadenoma right ovary (5.0 × 2.6 cm), left ovary normal, and moderate ascites with right-sided pleural effusion. Ascitic fluid tapping was done. Ascitic fluid was exudative in nature, negative for acid-fast bacilli, and cytology was negative for malignant cells.

In view of evidence of pleural effusion, ascitic fluid being exudative in nature and raised ESR, a strong possibility of pelvic tuberculosis (TB) was considered. In absence of bacteriological diagnosis, it was decided to give the patient a therapeutic trial of antitubercular treatment. The patient was started on Antitubercular treatment (ATT) – Rifampicin 450 mg, Isonizide 300 mg, Pyrazinamide 1125 mg, and Ethambutol 800 mg. After 10 days of starting ATT, the patient showed considerable response. Her appetite improved, weight increased, ascites decreased, and ESR and CA 125 also showed considerable reduction. After 10 days of ATT, the patient was discharged with advice to continue antitubercular drugs for 1 month. She followed up after completion of 40 days of ATT. There was a marked improvement in her general condition. Her appetite had improved and she had 5 kg of weight gain. CA 125 level was repeated and value was reduced to 12.2 mIU/L while ESR was reduced to 12 mm/h. The follow-up CT abdomen and pelvis showed no evidence of pleural effusion, ascites, or TO mass. Considering the improvement in all parameters, patient was put on ATT for a total of 6 months.

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DISCUSSION

CA 125 is a cell surface antigen expressed by derivatives of the coelomic epithelium (including endometrium) and is well established as a useful marker for the monitoring of the women with epithelial ovarian cancer. Moderate elevated levels (35–100 mIU/L) are often seen in endometriosis, early pregnancy, and acute pelvic inflammatory disease, besides several other benign conditions.[1] There are a few probable explanations for raised CA 125 levels in abdominal TB. Activation of inflammatory cascades due to mycobacterium TB may cause abnormal mesothelial cell proliferation, leading to elevated CA 125 levels. A second possible theory may be the similarity of certain surface antigens of mycobacterial cell membrane with epitopes of CA 125 tumor marker.[2]

Some cases of raised CA 125 in tubercular peritonitis have been reported previously. Thakur et al reported a case of a 48-year-old female diagnosed with TB peritonitis with increased CA 125 which was cured following antitubercular treatment, and the tumor marker level returned to normal.[3] Uzunkoy and colleagues reported of elevated levels of CA 125 in four abdominal TB patients. In another case reported by Tan et al, a patient finally diagnosed with peritoneal TB showed elevated serum CA 125 level mimicking advanced stage of ovarian cancer. Following ATT the symptoms resolved and CA 125 levels returned to normal.[4]

There have also been some cases with raised CA 125 in pulmonary TB. One such case of markedly elevated CA 125 levels in a woman with pulmonary TB, which returned to normal after ATT, was reported by Sulaiman and Tan in 2009.[5]

CONCLUSION

Raised CA 125 levels are generally seen in cases of ovarian malignancy. However, exceptionally high CA 125 may also be seen in patients with abdominal and pelvic TB especially in the context of Indian subcontinent.

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SHORT COMMUNICATION

Enhancement of Gentamicin Sensitivity in *Enterococcus faecalis* using Antidiabetic Molecule Gliclazide

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ABSTRACT

Enterococci, a low-grade pathogen, emerged as a potent nosocomial agent and have recently drawn the global attention because of resistance issues. To deal with this serious threat and reversal of drug sensitivity pattern, we made an attempt to sensitize the cells of *Enterococcus faecalis* with an oral hypoglycemic molecule gliclazide belonging to the class sulfonylurea. Interestingly, it was observed that results were quite encouraging as it was able to enhance gentamicin sensitivity by reducing the minimum inhibitory concentration (MIC). The decrease in MIC of gentamicin to *E. faecalis* is an indicator of reversibility of drug resistance. The findings have confirmed the concept that prior course or combination therapy of oral hypoglycemic drug with antibiotic gentamicin can be effective against Enterococci strains. However, auxiliary tests still need to be carried out further to understand the exact mechanism of the enhancement procured by gliclazide. The results have sowed the seeds of the concept of using gliclazide as a drug-resistant reversal molecule.

Keywords: *Enterococcus faecalis*, Gentamicin resistance, Gliclazide.

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INTRODUCTION

Enterococci, traditionally regarded as a low-grade pathogen, have recently drawn the global attention by being increasingly associated with nosocomial infections worldwide.[1] They are generally found to be associated with urinary tract infections, soft tissue infections, bacteremia, endocarditis, neonatal septicemia, and rarely meningitis. In the last decade, Enterococcus had stood out to be the second most common organism responsible for nosocomial infections after *Staphylococcus aureus*.[2] The reason behind the establishment of Enterococci as a prominent cause of hospital-associated infections is the organism's intrinsic resistance to all currently available cephalosporins and aminoglycosides.[3] Its capability of acquiring and exchanging genes encoding antimicrobial agent resistance also adds on to worsen the situation.[4] Enterococci isolated from clinical specimens were initially sensitive to treatment with aminoglycosides but, over the years they have started demonstrating high levels of resistance to both gentamicin and streptomycin generally mediated by aminoglycoside-modifying enzymes.[5] A common regime for serious Enterococcal infection like septicemia is the combination of cell wall inhibitors such as penicillin with aminoglycoside such as gentamicin.[6] The combination therapy of cell wall active agents and aminoglycoside usually results in the synergic killing of the organism. Agents interfering with the cell wall synthesis increase the uptake of aminoglycoside acting on the proteins involved in electron transport. The facultative anaerobic metabolism of *Enterococcus* imparts them a low-level resistance to all aminoglycosides. However, an increased resistance to gentamicin and streptomycin has started posing a threat of leaving us with no other combination of antimicrobial agents.[7-9] Thus, it has now become an absolute necessity to deal with the problem of multidrug resistance which has been neglected enough. With an effort to overcome the burning issue of antibiotic resistance, we made an attempt to sensitize the cells of *Enterococcus faecalis* with an oral hypoglycemic molecule gliclazide belonging to the class sulfonylurea. Gliclazide is known to binds to adenosine triphosphate (ATP)-dependent K^+ channel on the cell membrane which inhibits a tonic, hyperpolarizing efflux of potassium, it causes the electric potential over the membrane to become more positive and thus depolarizing the cell membrane.[10,11] The use of gliclazide would enhance the cell membrane permeability of the Enterococcal cells which consequently will have an effect on its sensitivity to the antimicrobial agent gentamicin.

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Fig. 1: The minimum inhibitory concentration of gentamicin for *E. faecalis* is 24 µg/ml of brain heart infusion

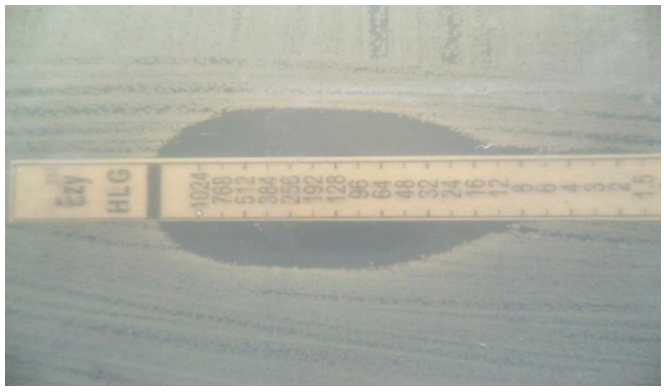

Fig. 2: The minimum inhibitory concentration of gentamicin for *E. faecalis* sensitized with gliclazide for 3 to 4 hours is 12 µg/ml of brain heart infusion

MATERIALS AND METHODS

Gentamicin was chosen as it is the most widely used aminoglycoside for the treatment of serious Enterococcal infections, normally in combination with cell wall active agents. *Enterococcus* strain used in the study was isolated from blood culture of a patient with bacteremia hospitalized at a tertiary care center. The isolated strain was identified as *E. faecalis* with the aid of traditional biochemical methods according to the scheme given by Facklam and Colins.[12] The isolate speciated to be *E. faecalis* was subjected to susceptibility testing by Kirby Bauer's disk diffusion method to the commonly used antibiotics at our hospital settings.[13] Gentamicin was the antibiotic which was chosen to be used further in our study. The minimum inhibitory concentration (MIC) of gentamicin was determined using high-level gentamicin strip (Hi-Media, Mumbai) by Epsilometer test (e-test) method. After the MIC value for gentamicin was obtained, strains were then sensitized using gliclazide (Tokyo Chemical Industry Co., Limited, Tokyo, Japan). The concentration of the gliclazide used was 0.05 mg/10 ml. A total of 10 ml of brain heart infusion (BHI; Hi-Media, Mumbai) broth was inoculated with the *E. faecalis* strain. To this BHI broth 0.05 mg of gliclazide was added and incubated at 37°C for 4 hours. The turbidity after 4 hours was matched with 0.5 McFarland's standard solution containing 10^5 colony-forming units/ml organisms. These sensitized (4 hours) cells were then subjected to E-test to determine whether there is any reduction in the MIC value. The same experiments were performed using 0.86% normal physiological saline containing 0.05 mg/ml gliclazide to check whether there is any difference in the sensitization on the cells of *E. faecalis* in reference to BHI. The controls contained BHI broth and 0.86% normal physiological saline inoculated with *E. faecalis* without gliclazide.

RESULTS

An attempt to check the effect of the drug on the cell membrane permeability of the bacterial cells and its consequent effect on its sensitivity to the antimicrobial agent gentamicin was carried out. Satisfactory findings have been obtained in this initial attempt. The initial (before sensitization) MIC of gentamicin with *E. faecalis* was found to be 24 µg/ml of BHI on high-level gentamicin strip (Hi-Media, Mumbai) under Epsilometer test (Fig. 1). After the sensitization of the microbial cells with gliclazide at the concentration of 0.05 mg/10 ml at 37°C for 4 hours, interestingly it was observed that MIC value of gentamicin for *E. faecalis* was dropped to 12 µg/ml of BHI (Fig. 2) on high-level gentamicin strip under Epsilometer test. Additional incubation of the cells with gliclazide at 37°C in 0.86% normal physiological saline for 4 hours promoted the drop of MIC further up to 8 µg/ml by *Epsilometer* test (Fig. 3). In sum, orally active antidiabetic molecule gliclazide has displayed as a potent molecule for enhancement of the activity of antibiotic gentamicin during killing of *E. faecalis*.

Fig. 3: The minimum inhibitory concentration of gentamicin for *E. faecalis* sensitized with gliclazide for 3 to 4 hours is 8 µg/ml of normal saline

DISCUSSION

A major reason behind survival of Enterococci in the hospital settings is its intrinsic resistance to the commonly used antibiotics and its ability to acquire resistance

to several others. To deal with this serious threat and reversal of drug sensitivity pattern, an attempt was made to enhance the drug sensitivity of *E. faecalis* for gentamicin by using an oral hypoglycemic drug gliclazide. The findings have confirmed the concept that prior course or combination therapy of oral hypoglycemic drug with antibiotic gentamicin can be effective against *E. faecalis*. However, auxiliary tests still need to be carried out further (undertaken in our laboratory) to understand the exact mechanism of the enhancement procured by gliclazide. It will be interesting to determine whether gliclazide can enhance the sensitivity of other antibiotics too. It is submitted that on an extensive literature search, such studies could not be found related to the present study. Though Sakharkar et al in their study used antibiotic combinations to enhance antibacterial efficacy and to prevent the development of resistance, natural products have been used for enhancing the sensitivity of a few antibiotics. The *in vitro* activities of antibiotic and physiochemical combinations against *Pseudomonas aeruginosa* were evaluated using the checker board assay and time-kill curve methods. There was synergism between gentamicin and caffeic acid and the MIC of gentamicin was 2 µg/ml. When gentamicin was combined with one-quarter of the MIC of caffeic acid, the MIC of gentamicin was reduced 4-fold. These results indicate the potential efficacy of phytochemicals in combination with antibiotics for enhancing total biological activity.[14] Reuk-ngam et al studied the synergistic effect of coronarian D (from the rhizomes of *Hedychium coronarium*) and different antibiotics on different bacteria. They saw best synergistic effect from the combination of coronarian D with gentamicin and coronarian D with oxacillin against *E. faecalis*. A concentration at 0.5 MIC coronarian D decreased the MIC of oxacillin and gentamicin in a range 16 to 260-fold.[15] Chaves et al found that the combination of ethanol extract of *Nasutitermes corniger* and gentamicin in *Escherichia coli* and erythromycin in *S. aureus* helped in reducing the MIC of the respective antibiotics.[16] Recently, Kristen et al reported the isolation of 3,4-dibromopyrrole-2,5-dione from Enterobacteriaceae and *P. aeruginosa*, from the marine bacterium *Pseudoalteromonas piscicida* and used for antibiotic activity enhancemen.[17] 3,4-Dibromopyrrole-2,5-dione which represents an inhibitor of Resistance-Nodulation-Division transporters decreased the MICs of two fluoroquinolones, an aminoglycoside, a macrolide, a beta-lactam, tetracycline, and chloramphenicol. Our results, i.e., effect of the oral hypoglycemic molecule gliclazide as prior treatment from gentamicin action on *E. faecalis in vitro*, were quite encouraging. It is able to enhance drug sensitivity probably through modulation of ATP-dependent K^+ channel on the cell membrane. The decrease in MIC of gentamicin to *E. faecalis in vitro* is an indicator of reversibility of drug resistance. The results have sowed the seeds of concept of using gliclazide as a drug resistance reversal molecule. Infections in diabetic patients are generally difficult to tackle. But the ability of oral hypoglycemic molecule gliclazide to help decrease MIC of gentamicin to *E. faecalis in vitro* is a unique observation. This concept may greatly benefit diabetic patients with *E. faecalis* infection.

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INTRODUCTION

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