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And the journey begins...

Prof. Dr. Madhab Lamsal

The explosion of knowledge has increased unlimitedly with the rapid expansion of the information technology. Now even the "common man" has access to this vast pool of knowledge and resources with several apps at their fingertips. However, there is a grave challenge in quality control and accessing the correct information from the vast pool of this bewildering knowledge. Research be it positive or negative needs to be published, else it will be perished, incurring a great loss to the global society. Publication has become easier these days, however, the challenges of publishing in reputed journals are still persisting.

Similarly, research is being done in every field and health sector is no exception. Any findings, even if apparently felt of less importance, paradoxically may be of greater value, depending on the community and the field concerned. The emergence and re-emergence of newer and older diseases pose yet another challenge. Thus, the research should find its place for publication in the right time in the right forum and should benefit people in general.

Clinical biochemistry is not restricted only to the diagnostic tools for metabolites, but can also be linked to molecular biology, proteomics, genomics and metabolomics, besides bioinformatics. It should find a rightful share also in therapeutics and individualized evidence based medicine.

Point of care testing has revolutionised the field of medical science with ample opportunities but creates a great challenge related to the quality services. Due to simplicity of operation, it may at times runs into the risk of displacing the role of qualified professionals. For example, the wide range of POCT devices in emergency setting may undermine the roles of biochemists in result interpretation as well as in overall patient management. Machines are intelligent but they cannot displace the human brain.

The machine equipment and reagent vendors should also be bound with high professional ethics so that the quality on the human health should not be compromised and jeopardised. The results obtained through laboratory investigation will be giving the quality not more than the input quality. In Nepal, due to paucity of up to date regulations, there has been the risk of converting the whole country into a dumping ground for plethora of health related equipment and risk of providing compromised services.

Within a short span of just two decades, the numbers of medical colleges and higher institutions imparting health related education in Nepal has multiplied significantly. The numbers of human resources in these fields and especially in clinical biochemistry has multiplied too. Thus, there has been a need of support and conduct science research and facilitate the collaboration of interdisciplinary clinical and biomedical research and publish them and disseminate them in the right forum.

Our objective of launching the ACCLM is to encourage quality research publications targeting the local and regional scientists in particular. The journey has thus begun, but it has miles to go, uninterrupted towards sustainability and that requires continuous input from various stakeholders, the researchers, the companies involved in diagnostics, the service-vendors, planners and society in particular by conducting quality research and feeding it with quality research publication. Let there be drain from the brain than having the brain in the drain.

Cytological and Biochemical Profile of Cerebrospinal Fluid from Meningitis Patients

Pinky Pandey,[1*] Bharat Jha,[2] Anima Shrestha[3]

BACKGROUND: The term Meningitis is used to describe an inflammatory infection of the membranes surrounding the brain and spinal cord, which occurs as either a primary disease or secondarily to disease in some other part of the body. The diagnosis is primarily confirmed by analyzing cerebrospinal fluid (CSF). Early diagnosis of the cause may be based on the cytological and biochemical parameters. Our objective was to determine the cytological and biochemical profile of CSF from meningitis patients.

METHODS: In this cross sectional study, a total of 356 CSF specimens were collected from patients suspected of meningitis and processed microscopically and microbiologically by standard microbiological methods in Emergency Lab of Tribhuvan University Teaching Hospital (TUTH) Kathmandu, Nepal, over a period of six months, from March to August 2014 to determine cytological and biochemical parameters.

RESULTS: Out of the 13 confirmed bacterial meningitis cases from 356 processed CSF samples, the mean value of total leukocytes count (TLC) was found to be 337.3 cells/mm^3 with predominant neutrophils (73.8%). The mean value of glucose and protein was 28.8 mg/dL and 89.4 mg/dL respectively in the cases of bacterial meningitis. Among the three confirmed cases of fungal meningitis, the mean value of TLC was 11.7 cells/mm^3 with lymphocytic predominance. In fungal meningitis glucose level was found to be normal (45.0 mg/dL) with slight increase in protein (48.7 mg/dL).

CONCLUSIONS: Bacterial meningitis is generally characterized by increased TLC with predominance of neutrophils, decreased glucose and increased protein levels whereas fungal meningitis displays decreased TLC predominance of lymphocytes, normal or reduced glucose and slight increased protein level.

Key words: Bacterial meningitis, biochemical and cytological parameters, fungal meningitis.

Introduction

The CSF is a clear bodily fluid that occupies the space between the arachnoid mater (meninges) and the pia mater. It is formed in the choroid plexus by both filtration and active transport. It protects the brain from the sudden change in pressure, it maintains stable chemical environment and removes wastes products of cerebral metabolism [1].

CSF evaluation is the single most important aspect of the laboratory diagnosis of meningitis. Analysis of the CSF abnormalities produced by bacterial, mycobacterial, and fungal infections may greatly facilitate diagnosis and direct initial therapy. Basic studies of CSF that should be performed in all patients with meningitis include measurement of pressure, cell count and white cell differential; determination of glucose and protein levels; Gram's stain; and culture [2].

The inflammation by various pathogens induces anatomical and physiological changes in the meninges which are responsible for characteristic changes in the values of CSF from patients with meningitis. The loss of integrity of cerebral capillaries and thus, the loss of integrity of the blood-brain barrier results in leakage of protein into the CSF and increased migration of Polymorphonuclear (PMN) leukocytes into the CSF [3].

Normal CSF contains 0-5 lecukocytes/mm^3, mainly lymphocytes, though in neonates cell count is up to 30/mm^3 [4]. WBC count of >500/mm^{3}with a preponderance of neutrophils is characteristic of a bacterial meningitis, and a WBC count of >100/mm^{3}with a preponderance of monocytes is characteristic of a viral meningitis a considerable pattern overlap is often found [5]. CSF glucose levels are used to distinguish bacterial meningitis (where it is usually decreased, usually <40 mg/dl) from aseptic meningitis (where the glucose levels are usually unaltered) [5]. Decreased CSF glucose results from changes in the physiological functioning of the choroid

[1]Department of Microbiology, St. Xavier's College, Maitighar, Kathmandu, Nepal, [2]Department of Biochemistry, Institute of Medicine, Tribhuvan University Teaching Hospital, Kathmanu, Nepal, [3]Department of Microbiology, Tri Chandra Multiple Campus, Kathmandu, Nepal

Correspondence to: Ms. Pinky Pandey, Department of Microbiology, St. Xavier's College, Maitighar, Kathmandu, Nepal.
Email: mercy.piku@gmail.com

epithelium as well as from consumption by bacterial pathogens and leukocytes [6]. Chemically meningitis can be differentiated from bacterial meningitis by CSF glucose levels (<10 mg/dL) and CSF WBC values (>7500 cells/mm^3) [7].

Proteins are largely excluded from the CSF by the blood - CSF barrier. Protein gaining access to the CSF primarily reaches the CSF by transport within pinocytotic vesicles traversing capillary endothelial cells [1]. Protein level greater than 200 mg/dL, is highly significant for bacterial meningitis indicating disruption of the blood-brain or the blood-CSF barrier [8].

This study aims to look at the changes in the cytological and biochemical value of CSF for the diagnosis of meningitis.

Methods

Across sectional study was carried out at the Emergency laboratory in Tribhuvan University Teaching Hospital (TUTH) from March to August 2014. Total 356 CSF samples were collected from patients clinically suspected of meningitis. The samples were processed microscopically and microbiologically to determine cytological and biochemical and microbiological parameters for diagnosing meningitis. The TLC were counted by Neubauer counting chamber method and differential leukocytes count (DLC) was by Wright's staining. The level of protein and glucose was determined by using Biochemistry Automatic Analyzer (Erba XL-200).

The specimens were cultured on Chocolate agar (CA), Blood agar (BA), MacConkey agar (MA), Mannitol salt agar (MSA), Nutrient agar (NA) and Sabouraud Dextrose agar (SDA). For bacterial isolates, BA and CA plates were incubated in candle jar (5-10% CO_2) at 37°C for overnight. MA plates were incubated at 37°C in incubator for overnight. SDA plates were used for culture of fungal isolates and incubated at 37°C for 2-3 days.

SPSS version 20 was used to analyze quantitative data.

Results

Among the total 356 processed CSF samples, there was a slight male dominance in the sex ratio (1.3:1) with males contributing 56.5% and females 43.5% (Table 1). The mean age of the patients was 27.8 years.

Among the total processed specimen (N=356), only 16(4.5%) cases were known to have laboratory confirmed cases of meningitis with the help of CSF culture results.

CYTOLOGICAL PROFILE

The maximum number (N=10, 13.5%) of isolates were isolated from the CSF samples having TLC >100 cells/mm^3 (Table 2).

And no isolate was from samples having normal cell count range (Table 2).

The mean value of TLC was found to be more (337.3 cells/mm^3) in bacterial meningitis compared to fungal meningitis (11.7 cells/mm^3). Neutrophils were predominant in bacterial meningitis whereas lymphocytes were predominant in fungal meningitis (Table 3).

BIOCHEMICAL PROFILE

The isolation rate of pathogens was highest (13 out of 72, 18.1%) in the CSF samples having glucose level <40 mg/dL. Similarly highest number of pathogens (13 out of 150, 8.7%) were isolated from the CSF samples having protein level >45 mg/dL.

Table 1. Age and Sexwise Distribution of Suspected Cases of Meningitis

Age Groups (Years)	Sex				Total	
	Male		Female			
	N	%	N	%	N	%
<1	21	5.9	16	4.5	37	10.4
1 - 14	56	15.7	45	12.6	101	28.4
14 - 30	47	13.2	37	10.4	84	23.6
30 - 60	49	13.8	42	11.8	91	25.6
> 60	28	7.9	15	4.2	43	12.1
Total	201	56.5	155	43.5	356	100

Table 2. TLC of Total CSF Specimen and Culture Positive Isolates

TLC (cells/mm^3)	Total CSF Samples (N=356)		Culture Positive Isolates (N=16)	
	Frequency (N)	Percentage (%)	Frequency (N)	Isolation Rate (%)
(Normal) 0 - 5	153	43.0	0	0
5 - 100	129	36.2	6	4.7
> 100	74	20.8	10	13.5

Table 3. Cytological Parameters in Different Types of Meningitis

Types of Meningitis/Cytological Parameters	Range	Mean	Standard Deviation
Bacterial Meningitis (N=13)			
TLC (cells/mm^3)	10 - 2000	337.3	523.8
DLC (%)			
Lymphocytes	5 - 46	26.2	12.9
Neutrophils	54 - 95	73.8	12.9
Fungal Meningitis (N=3)			
TLC (cells/mm^3)	10 - 15	11.7	2.9
DLC (%)			
Lymphocytes	100	100	0.0
Neutrophils	0	0	0.0

Table 4. Biochemical Parameters in Different Types of Meningitis

Types of Meningitis/ Biochemical Parameters	Range	Mean	Standard Deviation
Bacterial Meningitis (N=13)			
Glucose (mg/dL)	1.8-68.4	28.8	20.0
Protein (mg/dL)	46.9 - 175.6	89.4	42.6
Fungal Meningitis (N=3)			
Glucose (mg/dL)	30.6 - 72	45.0	23.4
Protein (mg/dL)	39.8 - 57.3	48.7	8.8

The mean value of glucose level (28.8 mg/dL) was found to be reduced than normal range and the protein level (89.4 mg/dL) was found to be above normal in bacterial meningitis cases whereas among the fungal meningitis, the mean value of glucose level (45 mg/dL) was found to be in normal range and protein level (48.7mg/dL) was slightly increased than the normal level (Table 4).

Discussion

Out of 356 CSF samples processed, only 16(4.5%) samples showed growth on CSF culture. This finding of culture positive result is in agreement with several similar studied conducted in Nepal. A study conducted in Manipal Teaching Hospital Nepal from 2000 to 2005 demonstrated 4.58% growth on CSF culture [9]. A similar study conducted from 2004 to 2008 showed 4.4% growth on CSF culture [10].

Among the 13 bacterial meningitis cases confirmed by culture results, it was observed that TLC was greater than normal range and found in the range of 10-2000 cells/mm^3 with predominant neutrophils (73.8%) in all cases, protein level were greater than normal value (89.4 mg/dL) and glucose contents were lower than normal range (28.8 mg/dL). Markedly decreased CSF glucose with markedly increased total protein, high WBC count with 89% Neutrophils, and the presence of a large number of PMN leukocytes and bacteria in the Gram-stained smear of the CSF sediment are the most striking laboratory results in bacterial meningitis [11].

Similarly, a study also mentioned that the examination of the CSF of a patient with acute bacterial meningitis characteristically reveals a cloudy fluid, consisting of an increased white blood cell count and predominance of PMN leukocytes, a low glucose concentration in relation to serum value, a raised concentration of

protein, and positive Gram stained smear and culture for the causative microorganism [12]. During the bacterial infection, due to microbial physiology, the protein is released and thus the level of protein is increased in CSF. The change in protein level than normal can be used to get the idea to distinguish viral from bacterial meningitis, as in bacterial infection, the protein level is usually raised in case of viral infection, the level of protein remains almost normal. Thus this finding in this study goes well with the established medical knowledge.

Among the fungal meningitis, mean value of protein level was slightly increased and glucose was within normal range. These findings are in accordance with other researcher's [13] finding which also displayed typical changes with elevated lymphocytes, elevated proteins (0.82 g/L), and decreased glucose levels (1.3 mmol/L).

Conclusion

Bacterial meningitis is generally characterized by increased TLC with predominance of neutrophils, decreased glucose and increased protein levels whereas fungal meningitis displays decreased TLC predominance of lymphocytes, normal or reduced glucose and slight increased protein level.

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I want to acknowledge all the staff of the Emergency Laboratory of TUTH for their help and cooperation during the laboratory work.

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Study of metabolic syndrome in postmenopausal women

Alina Shri Sapkota,[1*] Alisha Sapkota,[2] Kumananda Acharya,[1]
Mithileshwer Raut,[1] Bharat Jha[1]

BACKGROUND: Various studies throughout the world have documented higher prevalence of Metabolic Syndrome (MS) and CV risk factors in postmenopausal women. Abdominal obesity, a key component of metabolic syndrome is quite prevalent in South Asian women. However, studies that have investigated the effect of menopause on the health status of Nepalese women is lacking.

METHODS: This cross-sectional study was conducted in TUTH, Kathmandu Nepal. Forty five each premenopausal and postmenopausal (defined by cessation of menstruation for ≥12 months) women visiting the General Health Checkup Unit were enrolled as participants. Metabolic syndrome was defined by IDF criteria. BP, height, weight and WC were measured and BMI was calculated while fasting blood samples were analyzed for serum biochemical markers: FBG, lipid profile.

RESULTS: This study found higher prevalence of MS in postmenopausal women (57.8%) in comparison to premenopausal women (20%). 13.3% were diabetic, 23.3% were hypertensive, 82.2% had abdominal obesity (WC>80 cm), 43.3% were overweight (BMI 25-29.99 Kg/m^2) and 13.3 % were obese (BMI≥30 Kg/m^2). WC, BMI and SBP were significantly higher in postmenopausal group. Among the biochemical markers, FBG, TC, LDL-C, TG were significantly higher whereas HDL-C was significantly lower in postmenopausal women than in premenopausal women.

CONCLUSION: MS was highly prevalent in postmenopausal women. This is an indication of taking necessary preventive measures so that the risk of developing diabetes and cardiovascular disease can be minimized in the postmenopausal group.

Introduction

The presence of an adequate endogenous estrogen while the menstruation is regular in women is believed to have cardioprotective effect. Therefore, women during their fertile period of life are protected from coronary heart disease in comparison to men. However, following menopause, the ovaries permanently cease to produce estrogens and the female advantage in cardiovascular disease (CVD) present earlier would be lost. The decline in hormone level that begin few years earlier to menopause bring about various metabolic changes thereby worsening the CV risk profile. Metabolic disturbances including abdominal obesity, hypertension, fasting hyperglycemia, hypertriglyceridemia, low high-density lipoprotein cholesterol (HDL-C) etc. tend to cluster together. The constellation of these metabolic disturbances is termed as metabolic syndrome [1]. The metabolic syndrome is now considered to be driving the twin global epidemics of type 2 diabetes and CVD [2].

The declining level of estrogen and alteration of its ratio with testosterone has been implicated as a causal factor for the emergence of MS at menopausal transition [3]. Alterations in lipid metabolism with estrogen deficiency are thought to be a substantial component of CVD risk in postmenopausal women [4] but there are also direct effects of estrogen deficiency on body fat distribution (central obesity), insulin action, the arterial wall, and fibrinolysis that may influence cardiovascular risk. These factors contribute to an increased prevalence of the MS in postmenopausal women compared with premenopausal women [5].

According to the national report of Central Bureau of Statistics of Nepal in 2011, total population of Nepal is 26,494,504 out of which 13,645,463 are women. About 1.9 million of women were 50 years of age or older. Most of these women had or shortly would have their last menstrual period, thus becoming postmenopausal. As an average Nepali woman has life expectancy of 68 years, whereas in developed countries a lifespan up to 80 years is possible, which indicates that a woman spends one-third of her life after menopause [6].Out of several health problems occurring during this period, emergence of MS is

[1]Department of Biochemistry, Maharajgunj Medical Campus, Institute of Medicine, Kathmandu, Nepal, [2]National Public Health Laboratory, Teku, Kathmandu

Correspondence to: Alina Shri Sapkota, Department of Biochemistry, Institute of Medicine.
Email: alinasapkota12@gmail.com

of particular significance because there is substantial increase in metabolic disturbances. Globally, CVDs, often thought to be a 'male' problem, is the number one killer of women [7]. Thus, identification of postmenopa-usal women at high risk for MS has important implications for the reduction of CVD burden. CVDs are the consequences of mechanisms that are multifactorial in origin and MS includes almost all major factors for generation and progression of CVD. Therefore, the study of MS in postmenopausal women will be a single marker which can justify the multiple factors generating atherosclerosis, the cause of cardiovascular disease. International Diabetes Federation (IDF) criteria were employed for defining MS in this study.

The IDF consensus worldwide definition of the metabolic syndrome (2006) is:
Central obesity (defined as waist circumference with ethnicity-specific values*) and any two of the following [2]:

- Raised triglycerides: > 150 mg/dL (1.7 mmol/L), or specific treatment for this lipid abnormality
- Reduced HDL cholesterol: < 40 mg/dL (1.03 mmol/L) in males, < 50 mg/dL (1.29 mmol/L) in females, or specific treatment for this lipid abnormality
- Raised blood pressure (BP): systolic BP > 130 or diastolic BP >85 mm Hg, or treatment of previously diagnosed hypertension.
- Raised fasting plasma glucose (FPG): 100 mg/dL (5.6mmol/L), or previously diagnosed type 2 diabetes.

If FPG is >5.6 mmol/L or 100 mg/dL, an oral glucose tolerance test is strongly recommended, but is not necessary to define presence of the syndrome. If BMI is >30 kg/m², central obesity can be assumed and waist circumference does not need to be measure.

To meet the criteria, waist circumference must be: for Europeans, > 94 cm in men and > 80 cm in women; and for South Asians, Chinese, and Japanese, > 90 cm in men and > 80 cm in women. For ethnic South and Central Americ-ans, South Asian data are used, and for sub-Saharan Africans and Eastern Mediterranean and Middle East (Arab) populations, European data are used.

Methods

This cross-sectional study was conducted in Tribhuvan University Teaching hospital (TUTH). A total of 90 women visiting the OPD, for their general health check-up were randomly selected for the study. 45 of them were postmenopausal who had undergone natural menopause defined by cessation of menstruation for ≥12 months without any other medical cause and other 45 were regularly menstruating premenopausal women. Women who were amenorrhoeic due to hysterectomy or cessation of periods other than by a natural cause, women on HRT, women having irregular menses, pregnant and lactating women were excluded from the study.

An informed consent was obtained from each participant. After that a questionnaire was completed for each participant including demographic information, menopausal status, medical history and family history. The physical examination and clinical laboratory data was also noted.

BP measurement was done using a recently calibrated aneroid sphygmomanometer with an adequate cuff size, after participant had rested for at least five minutes. Weight was taken using a platform weighing scale. Standing height measurement was done with the participants barefooted, eyes looking ahead. Body mass index (BMI) was calculated by using the formula: (weight [kg]) / (Height [meter]^2). Using a flexible metric tape the waist circumference (in centimetres) was assessed at a point midway between the lowest rib and the iliac crest with the subject standing.

For biochemical analyses, 5mL of blood was drawn after an overnight fast (8-12hours) by venipuncture. Serum samples were separated, within half an hour, by centrifugationat1500-3000 rpm for 5min. Routine investigation was done on the same day of sample collection and included –blood glucose, TC, HDL-C, LDL-C and TG. Laboratory standard operation procedures were maintained for all laboratory analysis.

Determination of blood glucose was done by glucose oxidase peroxidise (GOD-POD) method, as described by Trinder, manufactured by Biolabo Reagents, France. Serum total cholesterol and triglyceride levels were determined enzymati-cally (Human, Germany). Serum HDL-C level

was determined enzymatically after precipitation of LDLs and VLDLs with dextran sulphate $MgCl_2$. LDL-C was calculated using the Friedewald formula, as follows.

LDL-C (mmol/L) = Total cholesterol - (Triglyceride/2.2 + HDL cholesterol)

When triglyceride concentration exceeded 4.5mmol/L, LDL-C was estimated by direct homogenous method (Biolabo, France). All the biochemical tests were performed by the fully automated chemistry analyzer, BT3000, Italy. Metabolic syndrome was defined by IDF criteria.

The data were entered in Microsoft Excel program (Microsoft office 2007). Statistical analyses were done by SPSS 20.0 version (Statistical Package for Social Science for Windows version; SPSS Inc., Chicago, IL). Mean comparison was done by t-tests.

Results

Out of total 90 participants in this study, 45 were regularly menstruating premenopausal women of age group 20-40 years. Remaining 45 were post-menopausal women who had cessation of menstruation at least 12 months earlier and they were of age group 45-70. Mean age of menopause was 48.9 years. Out of 90 participants 13.3% had the history of diabetes and 23.3% had the history of hypertension.

Discussion

Evidence of clustering of various cardio-metabolic derangements and CVD risk factors has been found in postmenopausal women in this study.

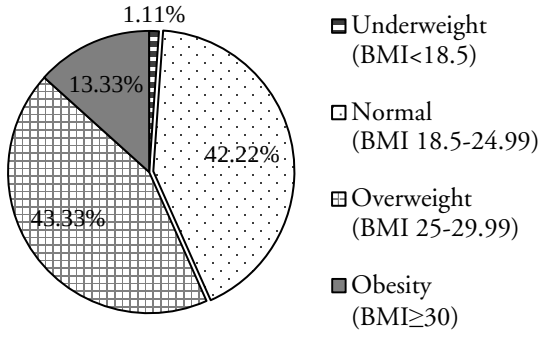

Fig. 1. Distribution of the study population based on BMI (n=90).

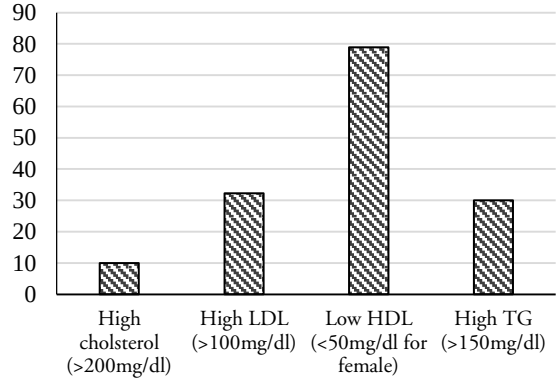

Fig. 2. Prevalence of Dyslipidemia (n=90).

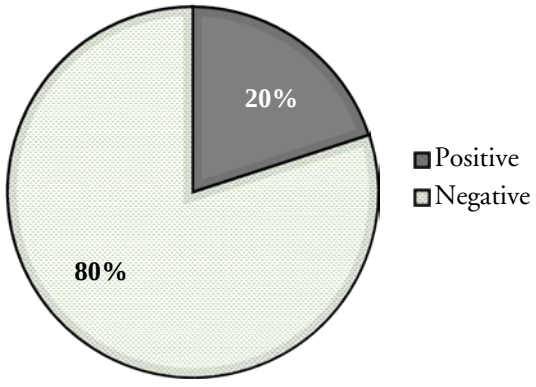

Fig. 3. Prevalence of MS in premenopausal (n=45).

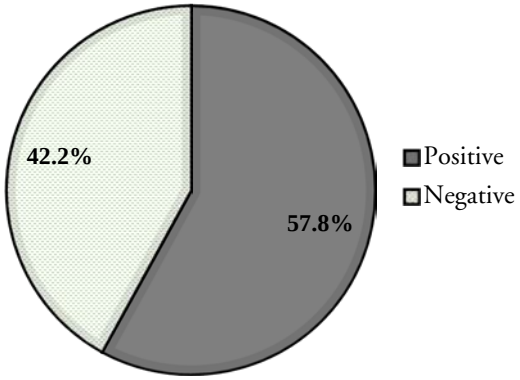

Fig. 4. Prevalence of MS in postmenopausal women (n=45).

Table 1. Comparison of Anthropometric and biochemical parameters between pre and post-menopausal women

Parameter	Premenopausal (n=45) Mean ± SD	Postmenopausal (n=45) Mean± SD	p value
Age (Years)	33.8 ± 5.7	57.9 ± 5.7	<0.001
SBP (mmHg)	112.5 ± 13.7	122.6 ± 12.1	<0.001
DBP (mmHg)	76.5 ± 9.8	79.4 ± 8.7	0.145
Height (cm)	154.8 ± 4.3	150.8 ± 5.5	<0.001
Weight (Kg)	60.2 ± 7.8	61.1 ± 10.1	0.589
BMI (Kg/m^2)	25.1 ± 3.4	26.9 ± 3.8	0.028
Waist Circumference (cm)	84.8 ± 9.6	94.3 ± 10.1	<0.001
Glucose (mmol/L)	4.6 ± 0.6	5.3 ± 1.2	0.002
Total cholesterol (mmol/L)	3.85 ± 0.7	4.21 ± 0.9	0.028
Triglygeride (mmol/L)	1.33 ± 0.6	1.69 ± 0.6	0.006
HDL-C (mmol/L)	1.12 ± 0.2	1.01 ± 0.2	0.021
LDL-C (mmol/L)	2.12 ± 0.6	2.43 ± 0.7	0.028

P-value <0.05 is statistically significant

The prevalence of MS was found to be 57.8% in postmenopausal and 20% in premenopausal women using the IDF criteria. Our findings were consistent with many of previous studies [8-13], where postmenopausal women were found to be at higher risk of MS than premenopausal women. The prevalence of MS has greatly varied across different studies. According to a study of Western India the prevalence of MS was 45% among premenopausal women, whereas it was 55% among postmenopausal women [11]. Eshtiaghiet. al., showed a prevalence of 53.5% MS in post-menopausal Iranian women, on the other hand it was only 18% in pre-menopausal women [10]. Differences in socio-environmental and genetic factors, lifestyles, type of menopause (natural/ surgical), time since menopause and criteria used for defining MS could be some of the reasons for this variability.

In the present study, significant differences in waist circumference and BMI among pre and post-menopausal women have been found and this is similar to some other studies [8, 14]. 82.2% of postmenopausal subjects are having abdominal obesity as defined by waist circumference >80 cm. Cross-sectional [15] and longitudinal studies [16] have shown that the menopausal transition is associated with a preferential increase in abdominal adiposity, independent of the effect of age and total body adiposity. In the present study abdominal obesity was also observed in 72.5% of premenopausal subjects. This could be due to various factors including physical inactivity, dietary habits, socioeconomic or genetic factor. This implies that abdominal obesity is the leading factor for metabolic syndrome. Current evidence implies that multiple risk factors for CVD emerge in the postmenopausal period, but features of the MS may be present even before menopause [17]. Moreover, South Asian Indians, in general, are prone to have MS at a younger age and have severe morbidity and mortality consequences as compared to Caucasians [18, 19].

While classifying the study population based on their BMI, only 1.1% were underweight, 42.2% had normal BMI, whereas, 43.3% were overweight and 13.3% were obese respectively. Altogether, 56.6% had higher than the normal BMI (≥ 25 Kg/m^2) and 82.2% of the women had raised WC (>80 cm). This is an alarming sign which indicates that lifestyle modification and dietary habit change is essential to control and prevent obesity and the consequences related to it.

In present study, a significantly higher level of metabolic risk factors including fasting blood glucose, total cholesterol, triglyceride and LDL-C were observed among post-menopausal women

than pre-menopausal women. HDL-C was also found to be significantly lower in the postmenopausal group. In agreement with the results of our study, many previous studies have reported higher prevalence of hypercholesterolemia [13, 14, 20, 21], hypertriglyceridemia [9, 13, 20-22], high LDL-C [8, 13, 20, 21], low HDL-C [20, 21] and elevated fasting blood glucose [13, 22, 23] among post postmenopausal women than pre-menopausal women. A high amount of abdominal fat is associated with increased insulin resistance, free fatty acid (FFA) levels, and decreased adiponectin. These factors contribute to increased secretion of apolipoprotein B (apo-B)-containing particles, leading to hypertriglyceridemia and increased hepatic lipase (HL) activity resulting in a predominance of small dense LDL particles and a reduction in large antiatherogenic HDL2 particles. A similar pattern of lipid abnormalities emerges with menopause [24]. Insulin resistance, with inadequate compensatory hyperinsulinemia, diminishes the normal suppression of FFA arising from adipose tissue by insulin. The increased levels of FFA may impair peripheral glucose uptake, increase hepatic gluconeogenesis, and reduce hepatic clearance of insulin [25].

In present study, a significantly higher level of systolic blood pressure was observed among post-menopausal women than pre-menopausal women (p=<0.001) whereas the mean diastolic blood pressure though elevated in post-menopausal group was not significantly different from the pre-menopausal group. Previous studies have reported higher prevalence of systolic blood pressure [13]. The decline in the oestrogen/androgen ratio dilutes the vasorelaxant effects of oestrogens on the vessel wall and promotes the production of vasoconstrictive factors such as endothelin [26]. Both male and female sex steroids have a regulating effect on the renin-angiotensin system (RAS) and affect angiotensinogen production and sodium metabolism. The decline in oestrogen levels around menopause causes an up regulation of the RAS with an increase in plasma renin activity [27]. Hypertension often clusters with other risk factors such as overweight, elevated insulin resistance, diabetes and lipid abnormalities.

Conclusion

MS was found high in postmenopausal women than in premenopausal women. Prevalence of MS was 57.8% in postmenopausal women and 20% in premenopausal group. Such a high prevalence of MS in postmenopausal group is an alarming sign. Prevention through changes in lifestyle, or early detection and treatment of elevated fasting blood glucose, hypertension, and hyperlipidemia are necessary for prevention of cardiovascular diseases in Nepalese women reaching menopause. Health professionals should consider the post-menopausal women as a major target group for prevention of MS, which is an underlying condition of many non-communicable diseases.

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Spectrum of acute leukemias diagnosed on flow cytometry: Analysis from tertiary care centre from North India

Surendra Koju,[1*] Man Updesh Singh Sachdeva,[2] Praveen Bose,[2] Neelam Varma[2]

BACKGROUND: Acute leukemias (ALs) are a heterogeneous group of malignancies with varying clinical, morphologic, immunologic, and molecular characteristics. WHO 2008 classification of ALs require a multi-parametric approach to the diagnosis. This study aims to evaluate the role of flow cytometry in diagnosis and sub-classification of acute leukemias.

METHODS: Consecutive patients of adult and paediatric ALs during June 2012 to May 2013 were retrospectively analyzed and studied using BD FACS Canto-II flow cytometer. The results of immunophenotyping were reviewed and analyzed for cross-lineage antigen expression.

RESULT: Over a period of year, 422 individuals were diagnosed as AL. There were 287 males and 135 females with M:F = 2.1:1. There were 237 adults & 185 children. 36.3% were AML and 60.4% were ALL, while 3.3% of cases were mixed phenotypic acute leukemia (MPAL). The commonest WHO subtype in AML group was AML with maturation being 31%. In case of ALL there were 83.9% B-ALLs and 16.1% T-ALLs. In MPAL B-Myeloid was 71.4%, whereas T-Myeloid was 28.6% of cases. Both AML and MPAL were more frequently seen in adults accounting to 83% and 92.9% respectively of all ALs cases. In contrast, 62% of ALLs were children and only 38% were adults. Out of all ALs, 37.6% of showed cross lineage antigen expression. In AML, B-ALL and T-ALL cross lineage antigen expression were 26.14%, 39.71% and 82.9% respectively.

CONCLUSION: Flow cytometry is useful in diagnosis and sub classification of AL. It is essential in cytochemical myeloperoxidase (MPO) negative cases. Cross- lineage antigen expression is frequent in ALs, and hence, lineage specific intra-cytoplasmic antibodies including anti-MPO and cytoplasmic-CD3 are essential for correct categorization of ALs.

Key words: Flow cytometer, Immuophenotyping, Acute leukemia

Introduction

Acute leukemias are a heterogeneous group of malignancies with varying clinical, morphologic, immunologic, and molecular characteristics. Flow cytometric immunophenotyping is a valuable tool for the diagnosis, classification, staging, and monitoring of acute leukemia. Differentiation between myeloid and lymphoid leukemias, most often made by flow cytometry, is important [1, 2]. Several advances in flow cytometry, including availability of new monoclonal antibodies, improved gating strategies, and multiparameter analytic techniques, have all dramatically improved the utility of flow cytometry in the diagnosis and classification of leukemia. Detailed understanding of phenotypic patterns of differentiation, particularly in myeloid leukemia, allows for more precise classification of leukemia than does morphology alone [3].

2008 World Health Organization (WHO) classification of hematolymphoid malignancy requires a multiparametric approach to diagnosis and outlines the morphologic, immunophenotypic, and genotypic features characteristic of each disease entity. Many of genetically distinct subgroups of leukemia have been found to be closely associated with distinct immunophenotypes. Thus, in addition to classification into differentiation-based subtypes, detailed flow cytometric studies can define complex antigenic profiles that are associated with specific molecular defects and well-defined biology. In summary, multiparameter flow cytometry is an invaluable tool in the diagnosis, classification, and monitoring of patients with acute leukemia.

The purpose of the study was to evaluate the role of flow cytometry in diagnosis and proper classification of acute leukemias. This study aims to find out the frequency of cross lineage antigen

[1]Department of Pathology, Dhulikhel Hospital-Kathmandu University Hospital, [2]Department of Hematology, Postgraduate Institute of Medical Education and Research, Chandigarh, India

Correspondence to: Surendra Koju, Department of Pathology, Dhulikhel Hospital-Kathmandu University Hospital P.O.BoxNo:11008, Kathmandu, Nepal. Phone number: 9841791258, E-mail: suren.koju@gmail.com

expression in AML, B-ALL and T-ALL.

Methods

Four hundred and twenty two patients of adult and paediatric acute leukemias, diagnosed in the Department of Hematology, PGIMER, Chandigarh, during June 2012 to May 2013 were retrospectively analysed. In addition to routine evaluation by complete blood counts, the patients were evaluated with peripheral blood films, bone marrow aspirate & trephine biopsy, using May Grunwald Giemsa, Hematoxylin & Eosin and cytochemical stains. Bone marrow aspirate and/or peripheral blood samples collected from all the patients were processed with standardized "lyse-stain-wash" technique, stained with 4 colors combination of antibody cocktails and acquired on dual laser BD FACS Canto II flow cytometer. All samples were processed within 24 hour of collection.

Immunophenotyping was done in mononuclear cell obtained by lysing whole blood by BD FACS lysing solution. For immunophenotyping various combination of flurochrome conjugated monoclonal antibodies (MoAbs) were added per tube in sample. They were conjugated with fluorescein isothiocyanate (FITC), phycoerythrin (PE), Allophycocyanine (APC) or peridinin chlorophyll protein (PerCP), and were directed to antigen of myeloid cell, B cell, T cell, monocyte or immature precursor cells. Data were acquired and blast gating strategy included using dot plots of CD45 expression versus intracellular complexity (side scatter angle, SSC) and also a second gate was based on cell forward scatter angle, (FSC) versus SSC.

Demographic profile of the patients, including age, sex was recorded for statistical analysis. Immunophenotypic profiles of all patients were analysed for cross lineage antigen expression.

Results

Over a period of one year, 422 individuals were diagnosed as acute leukemia. Diagnosis was based on morphology, cytochemistry and immunophenotyping by flow cytometer. There were 287 males and 135 females with M:F = 2.1:1 (Table 1). Fifty six percent (237/422) were adults & 43.8% (185/422) were paediatric cases (Table 2).

In 422 cases of acute leukemia, 36.3% (153/422) were classified as acute myeloid leukemia (AML) and 60.4 % (255/422) were acute lymphoblastic leukemia (ALL), while remaining 3.3% (14/422) of cases were mixed phenotypic acute leukemia (MPAL). The commonest WHO subtype in AML group was AML with maturation (FAB-M2) being 31% (47/153) (Table 3). In case of ALL there were 83.9% (214/255) B-ALLs and 16.1% (41/255) T-ALLs (Table 4). The most common subtype in MPAL was B-Myeloid, accounting for 71.4% (10/14), whereas T-Myeloid were 28.6% (4/14) of the MPAL cases. There was no case of B-T or tri-lineage MPAL (Table 5). AML was much more frequently seen in adults accounting to 83% (127/153) of all AML cases, and rest 17% (26/153) occurred in children. In contrast, 62% (158/255) of all ALL cases were children and only 38% (97/255) were adults. Similar to the distribution of AML, 92.9% (13/14) cases of MPAL were adults and only 7.1% (1/14) were children.

Out of 422 cases of acute leukemia 37.6% (159/422) showed cross lineage antigen expression. Out of 153 cases of AML, 40 cases showed cross lineage antigen expression. B lineage antigens were expressed in 22.5% (9/40), T lineage markers were seen in 75% (30/40) and both B & T lineage markers were present in 2.5% (1/40) cases. Similarly, in B-ALL, 85 out of 214 cases showed cross antigen expression comprising of myeloid lineage, T-lineage and combined myeloid-T-lineage cross expression in

Table 1. Frequency of type acute leukemias according to sex

Types of acute leukemia	Male (n= 287) n (%)	Female (n= 135) n (%)	Total (n=422) n (%)
Acute myeloid leukemia (AML)	89 (31%)	64 (47.4%)	153 (36.3%)
Acute lymphoid leukemia (ALL)	186 (64.8%)	69 (51.1%)	255 (60.4%)
Mixed phenotypic acute leukemia (MPAL)	12 (4.2%)	2 (1.5%)	14 (3.3%)

Table 2. Distribution of Acute Leukemias according to age group

Types of acute leukemia	Adult (n= 237)	Children (n= 155)	Total (n=422) n (%)
Acute myeloid leukemia (AML)	127 (83 %)	26 (17 %)	153 (36.3%)
Acute lymphoid leukemia (ALL)	97(38 %)	158 (62 %)	255 (60.4%)
Mixed phenotypic acute leukemia (MPAL)	13 (92.90 %)	1 (7.10 %)	14 (3.3%)

88.2% (75/85), 8.2% (7/85) and 3.6% (3/85) cases, respectively. Highest incidence of cross antigen expression was observed in T-ALL cases. Thirty four out of 41 (82.9%) of T-ALLs had myeloid, B-lineage and combined myeloid-B lineage marker expression in 41.2% (14/34), 41.2% (14/34) and 17.6% (6/34) cases, respectively (Table 5, Figure 1).

Discussion

Diagnosis of acute leukemia was tradionally based on morphological and cytochemical features (FAB classification) [4], but now, flow cytometry is also one of an indispensable tool for the proper classification and diagnosis of acute leukemia.

Table 3. Distribution of AML cases according to immunophenotyping

Types of AML	n=153 (%)
M0 (AML without differentiation)	10 (6.7%)
M1 (AML without maturation)	18 (12%)
M2 (AML with maturation)	47 (31%
M3 (Acute promyelocyticleukemia, APML)	23 (15%)
M4 (Acute myelomonocyticleukemia)	20 (13%)
M5 (Acute monocyticleuekemia)	23 (15%)
M6 (Acute erythroblasticleukemia)	2 (1.3%)
M7 (acute megakaryocytic leukemia)	6 (4%)
t-AML (Theraly related AML)	1 (0.6%)
AML-MDS (AML- Myelodysplastic syndrome)	1 (0.6%)

Table 4. Distribution of ALL cases according to Immunophenotyping.

Types of Acute Lymphoid Leukemia (ALL)	n= 255 (100%)
B-ALL	214 (83.90 %)
T-ALL	41 (16.10%)

Table 5. Distribution of MPAL according to immunophenotyping

Types of MPAL	n= 14 (100%)
B-Myeloid	10 (71.4%)
T-Myeloid	4 (28.6%)

Table 6. Frequency of abberent Cross lineage antigen expression in acute leukemias

Leukemia	No. of cases n=408	Abberant cases n=159
AML	153	40 (26.1%)
B-ALL	214	85 (39.8%)
T-ALL	41	34 (83%)

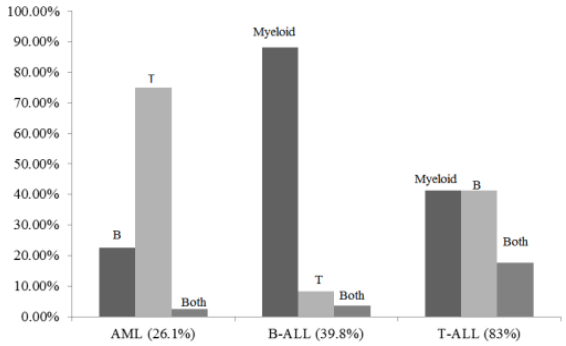

Fig. 1. Cross Lineage antigen expression in different acute leukemias.
B: B lineage cross Ag expression in AML and T-ALL.
T: Tlineage cross Ag expression in AML and B-ALL.
Myeloid: Myeloid lineage cross Ag expression in B-ALL and T-ALL.
Both: Combination of B & T, myeloid & T and Myeloid & B cross lineage Ag expression in AML, B-ALL and T-ALL, respectively.

Flow cytometry differentiate types of acute leukemia based on precursor cell expression of surface molecule that is called as cluster of differentiation (CD) antigen. Flow cytometry has also great importance in identification of biphenotypic leukemia and identification of unusual co-expression of antigen or abberent expression of CD antigen [5].

The core of monoclonal antibodies (MoAbs) investigated comprised: anti-MPO, CD13, CD33 and CD117 for the myeloid lineage cytCD3, CD2 and CD7 for the T-cell lineage, and CD19, CD10, CD20, and cytCD79a for the B-lymphoid lineage. All cases were also investigated for the expression of nuclear TdT, CD34, and HLA-DR and a substantial proportion for CD14, CD 64, CD 11c, CD41 and CD61.

Acute Myeloid leukemia is most common in adult whereas Acute lymphoid leukemia is common in children [6]. In our study also we observed the same frequency of acute leukemia based on age group.

In this present study, incidence of ALL was 60.4%, AML was 36.3% and remaining 3.3% was MPAL. In total AML cases, 15 % cases were APML while remaining 85% were non APML. Most of the other studies also found APML ranges as 5-14 %, and few study stated as 23% [7]. The commonest AML subtype was AML M2 that account for 31% which is similar to the study done by Ghosh et al [8].

Similarly, in case of ALL B-ALL (83.90%) is predominant to T-ALL (16.10%). Similar study was observed in the West, the predominant immunophenotype observed in ALL was B-ALL, accounting for 60-80% of total cases, whereas T-ALL comprised only of 15-20% [9]. In one study from eastern India, both T-ALL (50.4%) and B-ALL (49.6%) incidence was almost equal in distribution [10].

Biphenotypic acute leukemia or MPAL is a rare type of leukemia which probably arises from hemopoietic pluripotent stem cell with the capability of differentiating along both myeloid and lymphoid (T or B) lineages of antigen expression [11]. In our study, MPAL accounted for 3.3% (14/422).

In present study, the aberrant cross lineage expression of myeloid antigens on lymphoid leukemias was more common than expression of myeloid antigen on lymphoid leukemias. While in other study, cross lineage antigen expression of lymphoid lineage was common in AML (12).

Conclusion

Flow cytometry is useful in correct diagnosis and subclassification of acute leukemia and is essential in cytochemical myeloperoxidase (MPO) negative cases, as well as for the diagnosis of MPALs. In present study, majority of cases were cytochemically MPO negative (including B & T ALLs, AML-M0, M7 and MPALs). The incidence of AML and MPALs was higher in adults but ALL was predominantly seen in paediatric patients. Cross antigen lineage expression is a common phenomenon, most frequent in T-ALLs, and hence, lineage specific intra-cytoplasmic antibodies including anti-MPO and cytoplasmic-CD3 are essential for correct categorization of acute leukemias.

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The effects of Metformin Use on Body Mass Index: A Prospective Study

Tiwari S[1], Bhattarai A[2], Acharya RP[1], Prasad PN[1]

BACKGROUND: Limited number of studies has compared metformin with other Oral Hypoglycemic agents (OHAs) for reducing BMI and few of the results are controversial. Perhaps, this is of clinical importance because the Nepalese population presents different dietary habits in comparison with the European population. The objective of this study was to study the comparative evaluation of metformin with other OHAs influence on Body Mass Index (BMI) in Nepalese patients with diagnosed type 2 Diabetes Mellitus (T2DM)).

METHODS: A prospective cross sectional database of patients treated at diabetic clinic, TUTH, was analysed. Patients (N = 115) with type 2 Diabetes Mellitus and with complete BMI and HbA1c and treated with metformin and other OHAs, for at least three visits were included. Analysis of BMI and the type of oral agent was performed. Individuals were categorized as ideal weight, overweight, or obese (BMI <25, 25–29.9, and >30 kg/m^2, respectively).

RESULTS: There were differences between the values of BMI at presentation, the third, the sixth and the ninth months, between the metformin-treated groups compared to other OHAs treated groups. Metformin was given to 48 patients and OHAs to other 57 patients. In the metformin group, mean BMI decreased significantly during the treatment time, from 29.93±5.7 to 28.95±5.2 (<0.001). The obese the patients, the lower their BMI levels at the end of the analysis period. The mean BMI dropped by 0.9±1.18 in metformin group (from 29.93±5.7 to 24.83±3.6kg/m^2; p<0.001). It was found that the patients who had BMI higher than 30 kg/m^2 were significantly more likely to lose weight during the metformin therapy (p<0.05). However, the baseline change in body weight observed during metformin treatment correlated with the baseline metabolic control or its improvement during the analysis period.

CONCLUSION: Metformin use is associated with a significant decrease in body weight and BMI over long periods of time and it should remain a first choice drug for newly diagnosed T2DM patients, even more so for patients that are overweight or obese.

Introduction

Diabetes Mellitus (DM) is a chronic metabolic disorder characterized by the presence of hyperglycemia due to either a deficiency in the production or secretion of insulin, diminished tissue response to the actions of insulin, or both [1, 2]. Prevalence data indicate that diabetes has reached epidemic proportions worldwide, particularly in developed countries and emerging nations [3, 4]. The Nepal Diabetes Association reported that diabetes affects approximately 15% of people over 20 years and 19% of people over 40 years of age in urban areas of Nepal [5].

Although both type 1 and type 2 diabetes can potentially cause similar complications, the majority of diabetes related health care expenditures is spent on the treatment of complications in patients with T2DM [6]. Indeed, the vast majority of cases of Diabetes are T2DM [7].

T2DM has not just reached epidemic proportions worldwide. The number of affected individuals is increasing at a much faster rate than was originally predicted. Not surprisingly, the use of oral hypoglycemic agents used to treat this disease is also increasing rapidly.

Significant racial and ethnic disparities exist in the management of Diabetes [8, 9]. The aim of this study was to evaluate the effect of Metformin use on the BMI in Nepalese Diabetic patients. Nepalese are known to have a relatively lower BMI compared to Caucasians, Hispanics and African Americans. Our primary objective was to see whether Metformin affected the BMI significantly in Nepalese diabetics.

T2DM is treated with diet and exercise, coupled with oral hypoglycemic medications, insulin sensitizers, medications that impede hepatic production of glucose and prescribed insulin.

[1]Department of General Practice and Emergency Medicine, Tribhuvan University Teaching Hospital; [2]Department of Biochemistry, Institute of Medicine

Correspondence to: Aseem Bhattarai, Department of Biochemistry, Institute of Medicine, Kathmandu, Nepal. E-mail: dr.a.bhattarai@iom.edu.np

However, hypoglycemia, gastrointestinal side effects, weight gain, and lack of optimal control of postprandial glucose are limitations that may present with the use of these T2DM treatments, preventing patients from reaching glycemic control. Metformin is a medication that can significantly lower glycated hemoglobin (HbA1c), body weight, and postprandial glucose excursions in humans and significantly improve β-cell function. It has biological effects that slow gastric emptying and decrease appetite [10-14].

Till date, three biguanides have been used widely in patients with T2DM: metformin, phenformin and buformin; but only metformin remains part of today's worldwide pharmacopoeia. While there are close similarities between these drugs the unique properties of metformin explain its long lasting appeal. Metformin is of smaller molecular weight, more chemically stable, freely soluble in water and does not undergo substantial metabolism in vivo. Metformin acts by countering insulin resistance, which is thought to occur in principally in liver and muscle. Metformin has been used in the treatment of patients with T2DM since 1957 in Europe and 1995 in the Unites States. It is now the most commonly prescribed oral hypoglycaemic agent worldwide [13, 14].

In contrast to most other anti-diabetic drugs, Metformin often leads to modest weight reduction or weight stabilization. Due to its effects in suppressing the hepatic production of endogenous glucose and in increasing insulin sensitivity in adipose tissue and skeletal muscle, the agent is used particularly in T2DM and metabolic syndrome, in which insulin resistance is especially pronounced [13-16].

Although several studies clearly advocate the use of metformin as a drug of choice in patients that are overweight, both in terms of weight reduction as well as glycemic control, quite a few studies have shown ambiguity and the need for a study depicting our population couldn't be overemphasized at this point.

Our study intended to see if the use of Metformin as a choice of drug for T2DM was associated with a significant weight reduction in Diabetic patients.

Methods

A prospective cross sectional and non-interventional analysis was done following 115 patients in the out-patient and in-patient settings between January to October 2010. Both male and female diabetic patients aged 18 and above, among which, a total of 48 patients on Metformin and another 67 patients on other OHAs were included in the study.

The information collected included age, sex, weight, height, Blood Sugar levels and BMI. The BMI was documented at zero, three, six and nine months after metformin and other OHA use.

Results

At presentation, 14% (n=16) of the total selected patients (n=115) were in the normal weight category according to their BMI and a further 42% (n=48) and 44% (n=51) in the overweight and obese categories respectively.

Among the 48 patients using Metformin at the start of the study, 85% (n=41) were either overweight or obese. Incidentally, patients using other OHAs at the beginning of the study were mostly in the ideal weight category (58%, n=39).

The mean values of BMI between the two groups of patients were significantly different to start with, and further analysis revealed the mean values of BMI at three, six and nine months to be significantly different yet (P<0.001), as shown in Table 1.

Table 1. Mean Values of BMI across groups at different intervals

	Patients on Metformin	Patients on other OHAs	p-values (Pearson)
BMI at Presentation	29.93 ± 5.7	24.83 ± 3.6	<0.001
BMI (3 Months)	29.73 ± 5.5	24.89 ± 3.5	<0.001
BMI (6 Months)	29.29 ± 5.5	24.82 ± 3.5	<0.001
BMI (9 Months)	28.95 ± 5.2	24.69 ± 3.4	<0.001

The mean reduction in BMI at the end of the third month was not significantly different between the two groups of patients, but at the end of the sixth month and the ninth months, the mean BMI reduction achieved was significantly different between the two groups of patients. (0.63 ± 0.93 versus 0.00±0.7 at the end of the third month, $p < 0.001$ and 0.97±1.18 versus 0.13 ± 0.75 at the end of the ninth month, $p < 0.001$) (Table 2).

Table 2. Mean BMI reduction at different time periods

BMI difference at	Patients on Metformin	Patients on other OHAs	p-value
3rd month	0.19 ± 1.07	-0.06 ± 0.31	>0.05
6th month	0.63 ± 0.93	0.00 ± 0.7	<0.001
9th month	0.97 ± 1.18	0.13 ± 0.75	<0.001

Table 3. Mean BMI categorized at different Time Periods

BMI difference at	Ideal Weight	Overweight	Obese	p-values
3rd month	-0.048±0.78	0.03±1.43	0.43±0.67	>0.05
6th month	0.11±0.57	0.60±1.23	0.84±0.61	>0.05
9th month	0.23±0.39	0.71±1.39	1.46±0.86	<0.05

Based on their BMI, patients (on metformin) were grouped, as mentioned before into three categories (ideal weight, overweight and obese), and a comparison of the mean reduction in BMI values between the categories as seen in follow ups in the third, sixth and ninth months was done which showed that at the end of three months, a greater reduction in BMI was achieved among patients in the obese category, although, statistically, the results were not significant (mean reduction achieved was 0.43, p>0.05).

By the end of the sixth month, however, all patients showed a reduction in weight, with the patients in the obese category showing the greatest reduction and subsequently, BMI, but again, a statistical analysis did not reveal a strong association (p-value > 0.05).

At the end of the ninth month, however, on their third follow up, the reduction in BMI had increased in all categories, with patients in the overweight and obese categories showing a significant reduction (mean 0.71 and 1.46, respectively) in their BMI, with a strong statistical significance.

Further, an analysis of variance (ANOVA) was carried out to compare the mean reduction in BMI in patients and controls (in our case, patients on other OHAs), based on the different categories they fall under according to their BMI, this was cross tabulated against the different mean reduction levels in BMI achieved at the end of the third, sixth and the ninth months respectively, and the following results were obtained.

Thus, regardless of the time frame, the reduction of weight and subsequently, BMI, in patients on Metformin was always higher than that of the patients using other OHAs. At the end of the third month, however, the reduction achieved was not significantly different, but at the end of the sixth and ninth months, this difference was significant, as shown by Table 4.

Table 4. Mean BMI reduction according to BMI category between groups

BMI difference at	Ideal Weight		Overweight		Obese		p-values
	Patients on Metformin	Patients on other OHAs	Patients on Metformin	Patients on other OHAs	Patients on Metformin	Patients on other OHAs	
3rd month	-0.04 ± 0.78	-0.12 ± 0.32	0.03 ± 1.43	-0.01 ± 0.26	0.43 ± 0.67	0.13 ± 0.31	0.025
6th month	0.11 ± 0.57	-0.07 ± 0.57	0.60 ± 1.23	0.10 ± 0.87	0.84 ± 0.61	0.20 ± 0.86	0.001
9th month	0.23 ± 0.80	-0.07 ± 0.58	0.71 ± 1.39	0.39 ± 0.86	1.46 ± 0.86	0.62 ± 0.85	<0.001

Discussion

For the mean duration of the study (nine months), Metformin was given to 48 patients and OHAs to 57 patients. In the metformin group, mean BMI decreased significantly during the treatment time, from 29.93±5.7 to 28.95±5.2 (<0.001). Another notable figure was more obese the patient, the lower their BMI levels at the end of the analysis period. Mean BMI dropped by 0.97±1.18 in Metformin group (from 29.93±5.7 to 24.83±3.6; p<0.001). It was found that the patients who had BMI higher than 30 were significantly more likely to lose weight during the metformin therapy (p<0.05). However, the baseline change in body weight observed during metformin treatment correlated with the baseline metabolic control or its improvement during the analysis period. A decrease in BMI values was achieved independently of metabolic control. The antihyperglycemic efficacy of metformin was therefore somewhat dissociated from its weight-decreasing effect as shown by our study.

Although several studies have shown the advantage of Metformin in diabetic patients, [17] the observation periods were relatively shorter. Clarke and Campbell reported that Metformin monotherapy (n = 98) was equally effective as other OHA, on blood glucose control without

HbA1c measurements, and that metformin was superior in the body weight control in T2DM according to a prospective study done for a year [18].

The efficacy of Metformin has been well proven in the last several years [19]. In our study, Metformin was effective in reducing BMI significantly with an average decrease by 0.97±1.18 in kg/m^2 during the whole analyzed period. These results are in accordance with recent observations made by several authors, such as Hosokawa et al., [20] Garber et al., [21] and De Fronzo et al., [22] among others. However, the duration of these prospective studies did not exceed 6 months, which is considerably shorter than the period we analyzed. Given the outcome of our analysis we suggest that the Metformin is still efficacious, even though the maximum doses used in our patients did not exceed 2000 mg/day. We cannot comment, however, on any dose-response effect of Metformin, since the subjects included in our study did not change their dosage.

It is widely accepted that Metformin usually favors body weight loss [23]. Metformin treatment was associated in our patients with a small but statistically significant decrease in BMI, a mean of 0.97±1.18. It is worth mentioning that the most obese patients, whose initial BMI was 30 or more, lost during the analyzed period a mean of 1.46±0.86 kg/m^2. As the analyzed group consisted of ideal weight, overweight and obese subjects, we were able to reveal a significant positive association between the initial BMI and the subsequent decrease in BMI, i.e. the more patients weighed, the more weight they were likely to lose. This association may be explained by the fact that metformin induces a decrease in BMI in a proportional manner, usually allowing for 2–5% decrease [24].

We believe that the dissociation between metformin's impact on glycemic control and decrease in BMI requires further prospective studies, involving more homogenous groups of patients. It has also been established that metformin use is better than other OHAs in Nepalese T2DM [5]. The results of the study suggest that metformin be the drug of choice in treatment of T2DM whenever body mass is concerned, even more so in terms of the Nepalese population.

Conclusion

It can be concluded from the study that metformin is associated with a significant decrease in body weight and BMI over long periods of time and it should remain a first choice drug for newly diagnosed T2DM patients, even more so for patients that are overweight or obese.

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Microalbumin Status in Relation to Glycated Haemoglobin and Duration of Type 2 Diabetes Mellitus

Kumananda Acharya,[1*] Sangita Regmi,[2] Alina Shri Sapkota,[1]
Mithileshwer Raut,[1] Bharat Jha[1]

BACKGROUND: Diabetes mellitus (DM) is one of the most common endocrine disorders, characterized by hyperglycemia. Diabetic nephropathy is a consequence of long- standing diabetes and urinary microalbumin (Uma) status predicts progression to diabetic nephropathy. This study was conducted to know the status of Uma in relation to duration of diabetes and HbA1c level in patients with Type 2 diabetes mellitus (T2DM).

METHODS: This prospective cross-sectional descriptive study was conducted from July 1, 2014 to January 15, 2015 at TUTH, Kathmandu. Ninety-six known T2DM patients with age 35– 83 years were included in the study. EDTA venous blood and spot urine sample were collected for analysis of HbA1c and Uma respectively. Only those patients having HbA1c concentration ≥ 6.3% and duration of diabetes ≥ 6 months were included under the study.

RESULTS: Overall prevalence of microalbuminuria (MAU) was 39.6 %. MAU had a highly significant correlation with duration of diabetes ($r = 0.471$, $p<0.05$). Present study has shown positive correlation of MAU with HbA_{1c} level, although statistically insignificant ($r=0.245$, $p>0.05$).

CONCLUSIONS: Prolonged exposure to hyperglycemia-induced advanced glycosylation end products accumulations contributes for the development of MAU. So, duration of diabetes mellitus is main contributing factor for the development of MAU rather than HbA1c level alone. Screening for MAU to prevent renal impairment and measuring HbA_{1c} level on a regular basis for good glycemic control are important in diabetic patients.

Key words: Diabetes mellitus, Microalbuminuria, HbA_{1c}

Introduction

Diabetes Mellitus (DM) is a chronic, widely prevalent endocrine disease, which is characterized by hyperglycemia due to defects in insulin secretion, insulin action or both [1]. Diabetic nephropathy is most common complication of long-standing diabetes mellitus [2]. Persistent microalbuminuria (MAU) is the best predictor of progression to end-stage renal disease (ESRD) as well as cardiovascular complications [3]. Till date, different studies have been performed to find out the relationship between MAU, glycosylated haemoglobin (HbA_{1c}) and duration of diabetes. All the studies have not shown similar results and the relation between these parameters are not clear. This study was conducted to explore the underlying relationship between these parameters in our context.

Methods

This prospective cross sectional study was conducted on total 96 patients with T2DM. This study was carried out in Biochemistry Laboratory, Tribhuvan University Teaching hospital (TUTH) from July 1, 2014 to January 15, 2015. In this study, T2DM subjects having HbA_{1c} value >6.3%, who gave written consent were included under this study. Simultaneously, history of duration was taken and required informations were noted. HbA1c was estimated using NyCocard boronate affinity assay and Uma concentration was measured in spot urine sample using NyCocard immunometric assay. NyCocard Reader II was used for measurement of both HbA_{1c} and urinary microalbumin. Before performing microalbumin test, the urine sample was tested by uristrip method to exclude overt proteinuria from this study. MAU was diagnosed if albumin was between 20-200 mg/L.

Statistical analysis was done using SPSS version 17.0. Pearson's correlation was applied to observe associations of microalbuminuria with duration of diabetes and HbA_{1c} level. All p-values <0.05 were considered as statistically significant.

[1]Department of Biochemistry, Maharajgunj Medical Campus, IOM, TU, Kathmandu, Nepal; [2]Manmohan Cardiothoracic Vascular & Transplant Center, Institute of Medicine, TU, Maharajgunj, Kathmandu, Nepal

Correspondence to: Kumananda Acharya, Maharajgunj Medical Campus, Institute of Medicine, Tribhuvan University, Kathmandu, Nepal. E-mail: shinning_kuma@iom.edu.np

Results

A total of 96 patients 54 males and 42 females were included in this study. Overall prevalence of MAU in the present study was 39.6 % (38/96). Among total 54 males, prevalence of UMA was 44.4% (24/54). And among 42 females, prevalence of UMA was 33.3 % (14/42). Mean age of patients with UMA was 61.42±7.45 years and in normoalbuminuric patients it was 59.21±8.49 years.

Duration of diabetes ranged between 6 months and 18 years. Out of total 96 patients, 52 had duration of diabetes <5 years and among them 18 (34.6 %) had MAU. Twenty six had duration of diabetes ≥5 to 10 years, among them 10 (38.5 %) had MAU. Twelve had duration of diabetes ≥10 to 15 years, among them 6 (50.0 %) had MAU, and six had duration of diabetes ≥15 years, among them 4 (66.7 %) had MAU [Table. 1]. Mean duration of diabetes in microalbuminuric patients was 7.71±5.65 years while in normoalbuminuric patients it was 5.17±4.32 years, which was statistically significant. Pearson correlation analysis showed statistically significant correlation of MAU with duration of diabetes (r= 0.471, $p < 0.05$) [Table 3].

Out of total 96 patients, 60 had HbA_{1c} level < 8.0 % , among them 20 (33.3 %) had MAU. 20 had HbA_{1c} level ≥ 8.0 – 10.0 % , among them 10 (50 %) had MAU. 12 had HbA_{1c} level ≥ 10.0–12.0 % , among them 6 (50 %) had MAU 4 had HbA_{1c} level ≥ 12.0 % , among them 2(50%) had MAU [Table 2]. Mean HbA_{1c} level in microalbu-minuric patients was 8.63±1.89 % while in normoalbuminuric patients it was 8.02±1.77 %. Although MAU positively correlated with HbA1c but statistically was insignificant. Pearson correlation analysis did not show statistically significant correlation of MAU with HbA1c level (r= 0.245 $p > 0.05$) [Table 3].

Table 1 :Prevalence of microalbuminuria in relation to duration of T2DM

			Group for Uma		Total
			Normoalbuminuria	microalbuminuria	
Duration (years)	<5 years	N	34	18	52
		Percent	65.4%	34.6%	100.0%
	≥5-10 years	N	16	10	26
		Percent	61.5%	38.5%	100.0%
	≥10-15 years	N	6	6	12
		Percent	50.0%	50.0%	100.0%
	≥15 years	N	2	4	6
		Percent	33.3%	66.7%	100.0%
Total		No.	58	38	96
		Percent	60.4%	39.6%	100.0%

Table 2:Prevalence of microalbminuria in relation to HbA1c level in T2DM

			Group for Uma		
			Normoalbuminuria	Microalbuminuria	Total
HbA1c level (%)	<8	N	40	20	60
		Percent	66.7%	33.3%	100.0%
	≥8-10	N	10	10	20
		Percent	50.0%	50.0%	100.0%
	≥10-12	N	6	6	12
		Percent	50.0%	50.0%	100.0%
	≥12	N	2	2	4
		Percent	50.0%	50.0%	100.0%
Total		N	58	38	96
		Percent	60.4%	39.6%	100.0%

Table 3: Correlation microalbuminuria with duration and HbA1c level in T2DM

Variable	Normoalbuminuria	Microalbuminuria	P value	Correlation coefficient (r)
Mean duration (years)	5.17 ± 4.32	7.71 ± 5.65	0.001*	0.471
Mean HbA1c level (%)	8.02 ± 1.77	8.63 ± 1.89	>0.05 **	0.245

*Statistically significant
**Statistically insignificant

Discussion

The aim of present study was to explore the prevalence and associations of MAU with different parameters in T2DM in our context. Present study has shown overall prevalence of MAU to be 39.6 %. Wu et al has reported slightly higher prevalence of MAU (39.8 %) in Asian population, [4] whereas, another Asian study has shown 36.3% MAU in T2DM Indian population [5] The overall prevalence of MAU in present study is higher than the study done in Kathmandu valley, Nepal by Maharjan et al, [6] which was 36.79% but was lower than the study done in Pokhara, Nepal by Sigdel et al, [7] which was 45.5%. Prevalence of MAU was reported 25 % in one study conducted by Ghai et al. [8]. This marked variation in results for prevalence of MAU might be due to sample size, sample selection, study design, hypertension, poor glycemic control, duration of diabetes, age and gender structure of study population.

Present study has shown significant correlation of MAU with duration of diabetes which is in accordance with many previous reports [9]. Chowta et al showed statistically significant correlation of MAU with duration of diabetes ($r = 0.839$, $p < 0.0001$) [10].Naz et al had reported similar type of result in patients from Islamabad and Rawalpindi [11].

This study has shown positive correlation of MAU with HbA1c, but statistically insignificant ($r = 0.245$, $p > 0.05$). In a study conducted by Maharjanet al in Kathmandu valley, Nepal comparison of HbA1c level between microalbuminuric and normoalbuminuric was not statistically significant [6].ShonimaVenugopal and Uma M lyer showed statistically significant correlation of UMA and HbA1c level ($p < 0.05$) [12].Manjrekar et al has reported gradual increase in prevalence of MAU with similar increase in HbA1c level [13].Similarly, Gupta et al performed an independent study and reported strong association of HbA1c level with urinary microalbumin excretion [14]. The difference in results may be due to limited sample size. Hence, further study with a larger sample is necessary in order to confirm the result obtained in present study.

This study shows conclusive evidence that urinary microalbumin excretion was significantly correlated with duration of the disease and level of HbA1c positively correlated with urinary microalbumin although statistically insignificant. Duration of diabetes contributes for the development of MAU and then diabetic nephropathy by prolonged exposure to hyperglycemia induced advanced glycosylation end products.

Conclusion

Overall prevalence of microalbuminuria in T2DM was 39.6%. Urinary microalbumin excretion correlated significantly with duration of diabetes. Increased HbA1c level positively correlated with MAU, although statistically insignificant. Regular screening for urinary microalbumin as well as HbA1c and good glycemic control is recommended in such patients.

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Interference of Bilirubin in Creatinine Value Measurement by Jaffe Kinetic Method

Siddhartha Shankar Chaudhary,[1*] Jay Prakash Shah,[2] Ram Vinod Mahato[3]

BACKGROUND: Creatinine measurement in icteric sample is a major but unresolved problem. Bilirubin causes negative interference in creatinine value measurement using general techniques. The objective of this study was to find differences in creatinine value by Jaffe Kinetic method pre-incubation and without pre-incubation with NaOH.

METHODS: This was a cross sectional, descriptive study carried out in 71 samples with different level of bilirubin concentration. We took blood samples of 71 different patients, 48 males and 23 females, from two different hospitals of Kathmandu village. Both creatinine and bilirubin concentration in serum samples were measured by using Staxfax 3300 semi auto analyzer in the hospital. In the laboratory creatinine value was measured by kinetic method and bilirubin measured by Jendrassik/ Grof method using commercial kits. Statistical analysis of quantitative data was done by using SPSS version 16.0.

RESULTS: The results shows differences in creatinine values with respect to methods and extent of bilirubin concentration. It was found that the creatinine obtained by pre-incubation with NaOH has greater value than without pre-incubation (i.e. by direct estimation using working reagent). It was also shown that the high bilirubin cause the interference in greater extent. The significant interference was seen in the sample with bilirubin concentration greater than 20 mg/dl i.e. creatinine value after treatment with NaOH prior to dispensing picric acid is significantly increases, $P<0.01$ at 99% confidence level.

CONCLUSION: This shows that the bilirubin has negative interference in creatinine value measurement by ordinary laboratory practices and interference increases with higher concentration of bilirubin in blood sample.

Key Words: Creatinine. True creatinine, Bilirubin total, Bilirubin direct (T/D), out value

Introduction

Creatine is present as creatine phosphate, reserved energy in muscle. Creatine is produced in kidney, liver and pancreas by enzymatic reactions. Thus produced creatine is transported to different organs such as brain and muscles where it is phosphorylated to form creatine phosphate and stored as reserved energy source. Creatinine is a breakdown product of creatine phosphate and produced in a fairly constant rate by the body depending on muscle mass, age, sex, diet and exercise [1]. It is a waste product excreted through urine hence used as a helpful parameter to measure clearance test of GFR. Generally it is fairly constant but also found increased with certain diets. The serum creatinine level is abnormal in cases of muscle and kidney diseases. Its estimation occurs as an important biochemical parameter in clinical laboratory tests.

Bilirubin is a yellow pigment formed inside the body by the metabolism of heme. It is formed by the enzymatic reduction of biliverdin to bilirubin. It is also an important biochemical parameter particularly of liver function tests. These tests are intertwined in combined hepatic and renal test. The combined liver and kidney function test provide the knowledge liver and kidney disease that occur in the same patient [2]. Creatinine estimation in such condition should be done with great care since bilirubin has negative interference on creatinine measurement by Jaffe Kinetic method.

The exact mechanism of bilirubin interference is not known but the color of bilirubin effects on spectrum absorption with yellow color of picrate used in creatinine measurement [3]. In the case when creatinine has to be measured in icteric sample (high bilirubin) then color produced by bilirubin should be removed or minimized. This can ordinarily be done by oxidation of bilirubin to biliverdin by oxidizing agents. In this study oxidation of bilirubin is carried out by pre-incubation with NaOH before estimation of creatinine [4].

[1]Department of Medical Biochemistry, Nobel College, Sinamangal, Kathmandu, Nepal. [2]School of Health and Allied Sciences, Pokhara University, Pokhara. [3]Central Campus of Technology, Hattisar, Dharan.

Correspondence to: Department of Medical Biochemistry, Nobel College, Sinamangal, Kathmandu, Nepal.Email: ch_siddharth07@yahoo.com, Mobile: +977 9841585427

Methods

The laboratory based, descriptive, cross-sectional study was carried out in 71 serum samples taken from patients visiting two different hospital in Kathmandu valley. The serum samples contain different concentration of bilirubin. Study population were jaundiced and non-jaundiced patients attending OPD and wards of the hospitals. The study was conducted for three months from August 2010 to November 2010. Laboratory investigation of the samples were carried out by using Staxfax 3300 semi auto-analyzer..

Blood samples were collected purposively both icteric and normal samples during the study period. Approval from the institution and patients' consent were taken before conducting study and sample collection. Biochemical parameters were creatinine and bilirubin serum concentration and variables were age and sex of the patients.

The estimation of biochemical parameters were carried out by using Staxfax 3300 semi auto-analyzer. Venous blood was collected by using tourniquet and was kept in a test tube. The clotted blood sample was quickly centrifuged and separated serum was used for the estimation of creatinine and bilirubin value. CREST BIOSYSTEMS, Jaffe Kinetic method, creatinine kit was used for serum creatinine measurement.

SERUM CREATININE MEASUREMENT BY JAFFE KINETIC METHOD:

Creatinine reacts with picric acid in an alkaline medium (i.e. alkaline picrate) to form an orange colored complex. The intensity of the color formed during the fixed time is directly proportional to the amount of creatinine present in the sample. And creatinine is measured kinetically at 490nm (490-510).

Creatinine+ Alkaline picrate → Orange colored complex

SERUM BILIRUBIN (TOTAL/DIRECT) MEASUREMENT BY MODIFIED JENDRASSIK/ GROF METHOD:

Bilirubin reacts with diazotized suphanilic acid (DSA) to form a red azo dye. The absorbance of this dye at 546 nm is directly proportional to the bilirubin concentration in the sample. Water soluble bilirubin glucuronides react directly with DSA whereas the albumin conjugated indirect bilirubin will only react DSA in the presence of an accelerator: total bilirubin = direct + indirect

Sulphanilic acid + Sodium nitrite → DSA

Bilirubin + DSA → Direct Azobilirubin

Bilirubin + DSA + Accelerator → Total Azobilirubin

Statistical analysis of data was carried out by using statistical package SPSS version 16.0 and Microsoft excel.

Results

The analysis of the data showed that the mean concentration of bilirubin was found to be 5.728 and that of creatinine 1.048. The mean difference of creatinine (without pre incubation) and true creatinine (pre incubation with NaOH) was found to be statistically significant, i.e $P<0.01$ [Student's paired t-test (1 tail)]. The creatinine value was found to be decreased in procedure without pre incubation.

The maximum and minimum bilirubin values were 27.10 and 0.70 mg/dl respectively. Thus this study covers a required range of icteric sample. The minimum and maximum creatinine value obtained were 0.00-3.90 mg/dl in this study.

Discussion

In the present study we find that bilirubin interfere in the estimation of creatinine by Jaffe Kinetic method. For normal bilirubin also creatinine is found to be slightly decreased but is not significant. The creatinine value obtained by pre incubation with NaOH (i.e true creatinine) is found to be increased than creatinine obtained without pre incubation (creatinine) (Table1). Similar findings were reported by R.Vaishya, S.Arora et al, 2010.

The mean value of creatinine was different for pre-incubation with NaOH and without pre-incubation (Table 2). Little variation in creatinine estimation for normal bilirubin concentration. For bilirubin concentration <1mg/dl there was just little variation in creatinine value.

Table 1. Mean, median, S.D, min/maximum value of bilirubin and creatinine of total (71) samples

	Bilirubin (mg/dl)	Creatinine (without pre-incubation (mg/dl)	Creatinine (pre-incubation with NaOH) (mg/dl)	t-test (1-tail)
Mean	5.72	1.04	1.3	
Median	2.80	0.9	1.1	
SD	6.42	0.67	0.67	<0.001
Minimum	0.70	0.0	0.6	
Maximum	27.10	3.9	4.1	

Table 2. Different bilirubin concentrations and their respective findings

Bilirubin (mg/dl)		Creatinine without pre-incubation (mg/dl)	Creatinine with NaOH pre-incubation (mg/dl)	t-test (1-tail)*
	N	22	22	<0.001
< 1	Mean	0.86	0.97	
	S.D	0.21	0.23	
	N	24	24	<0.001
1-5	Mean	1.19	1.37	
	S.D	0.74	0.75	
	N	12	12	<0.001
5-10	Mean	1.15	1.44	
	S.D	0.56	0.53	
	N	5	5	<0.01
10-15	Mean	1.74	2.16	
	S.D	1.20	1.13	
	N	5	5	<0.01
15-20	Mean	1.05	1.62	
	S.D	0.72	0.70	
	N	3	3	<0.01
> 20	Mean	0.0	1.0	
	S.D	0.00	0.28	

*Value of Student's paired t-test between creatinine (without pre incubation) and true creatinine (pre incubation with NaOH).

Similarly for bilirubin concentration 1-5mg/dl and 5-10mg/dl there was mild increase in creatinine value (pre incubation with NaOH) and moderate increase for bilirubin concentration of 10-20mg/dl (in two categories 10-15 and 15-20 mg/dl). The significant increase in creatinine concentration after pre-incubation was found for bilirubin concentration >20mg/dl.

In this study bilirubin concentration >20mg/dl gave "out value" when creatinine is measured without pre incubation with NaOH. Though it is different from R.Vaishya, S.Arora et al but satisfied with other researchers: bilirubin- no significant interference up to 20mg/dl (Beckmen coulter).

The sample size in this study was 71, may not cover the large number of icteric samples but, this sample size was sufficient to give knowledge of bilirubin interference in creatinine estimation. Due to the scarcity of enough icteric samples this study did not contain large number of samples with bilirubin concentration higher than 20 mg/dl.

This study was carried out to remove bilirubin interference only but there might be other interferents like acetoacetate or lipids in the same sample that could cause the similar negative interference in creatinine estimation in Jaffe Kinetic method [5].

Creatinine estimation is primarily used indicator for renal function. As shown by the results of this study creatinine estimation by normal method may give false value of creatinine [6]. Hence care should be taken before creatinine value measurement in icteric sample particularly bilirubin > 5 mg/dl.

Conclusion

According to this study pre-incubation with NaOH helps to reduce this negative interference

of bilirubin in creatinine value measurement by Jaffe Kinetic Method.

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Lipid profile in patients with alcohol dependence syndrome

Mithileshwer Raut,[1*] Prashant Regmi,[2] Saroj Prasad Ojha,[3] Bharat Jha[1]

BACKGROUND: Alcohol dependence syndrome (ADS) has become a global public health challenge because of its high prevalence and the concomitant increase in risk of liver disease, cardiovascular disease and premature death. Influence of alcohol use on lipid metabolism is well recognized. Investigations had been carried out in the earlier period on abnormal lipid profile as a risk factor for Coronary Heart disease (CHD). Patients of alcohol dependence usually have a consumption pattern of more heavy use. Therefore it is useful to study the lipid profile in patients of alcohol dependence, to understand the effects of increasing levels of consumption.

METHODS: This cross-sectional study was conducted in TU Teaching Hospital. ADS patients were screened by the consultant psychiatrist using the Alcohol Use Disorder Identification Test (AUDIT) questionnaire. A total of 89 patients scored positive on the AUDIT as having alcohol-related problems and were included in the study. 89 ADS patients and 89 healthy controls both male and female were enrolled as participants. Blood Pressure and other anthropometric parameters were measured while fasting blood samples were analyzed for serum lipid profile. SPSS program was used to analyze data, t-test & Spearman's correlation coefficient was used to find correlation.

RESULTS: Among the ADS cases 95% were current smokers. Mean age of cases and controls was 35.42±5.6 & 34.53±3.5 years respectively. The mean total cholesterol levels were found to be higher in cases (5.41±0.70) than controls (3.79±0.74) with a strong statistical significance ($p<0.001$). Also, Mean triglyceride (TG) levels (2.09±0.72), along with the mean HDL-cholesterol (1.66±0.40) and LDL-cholesterol levels (2.79±0.81) were also elevated in cases when compared to the control samples ($p<0.001$).

CONCLUSION: This study has demonstrated definitive lipid profile changes in patients of alcohol dependence, with some correlation to the liver dysfunction. Alcohol causes alteration in various parameters of lipid metabolism including those which predispose to CHD. Low to moderate alcohol use over prolonged periods has been linked to have protective influence for development of coronary heart disease (CHD), through increase in high density lipoprotein cholesterol (HDL-C) levels.

Key words: ADS (Alcohol dependence syndrome), CHD (Coronary heart disease), HDL-C (high density lipoprotein cholesterol), TG (Triglyceride), LDL-C (Low density lipoprotein cholesterol)

Introduction

Alcohol consumption might be the cause of several diseases, and it is, well known, the high burden of its consumption over mortality around the world [1]. Alcohol is the only psychoactive drug that provides energy (7.1 kcal/g). However, its calories are considered "empty," because alcohol ingestion does not provide vitamins and minerals [2] and its use may cause alterations to the nutritional state [3]. Alcohol dependence syndrome is defined as "A cluster of physiological, behavioral, and cognitive phenomena in which the use of a substance or a class of substances takes on a much higher priority for a given individual than other behaviors that once had greater value [4]. Hazardous alcohol intake and related disorders are a major health issue. A World Health Organization (WHO) project on psychological problems in general practice has shown that alcohol dependence or harmful alcohol use is present in about 6% of patients attending primary care, ranking third in frequency after major depression and generalized anxiety [5]. Harmful or heavy alcohol drinking makes a substantial contribution to the burden of disease and premature mortality [6]. Among persons admitted to general hospitals, 20 to 40 percent have alcohol-related problems, and among the elderly, alcohol-related hospitalizations are as numerous as those due to myocardial infarction [7]. The liver is the organ most severely affected by alcoholism. In some urban areas, cirrhosis (usually a complication of alcoholism) is the fourth most frequent cause of death among people 25 to 64 years of age [8]. Different studies have been done in alcoholic liver diseases but only few studies have been done in alcohol dependence syndrome. Alcohol abuse is a major

[1]Department of Biochemistry, Maharajgunj Medical campus, IOM, TU, Nepal. [2]Nepal Medical College, Jorpati, Kathmandu, Nepal. [3]Department of Psychiatry and Mental Health, TU teaching hospital, Nepal
Correspondence to: Mithileshwer Raut, Department of Biochemistry, Maharajgunj Medical Campus, IOM, TU
Email: clinbio.mraut@gmail.com

health problem as well as social problem in the community. Patients of alcohol dependence usually have a consumption pattern of more heavy use. Therefore it is useful to study the lipid profile in patients of alcohol dependence, to understand the effects of increasing levels of consumption. Alcoholism may lead to the different health consequences, like alcoholic liver diseases, cardiovascular diseases, and kidney diseases. So, the early diagnosis of ADS can help the patient to prevent from these major health problems.

Methods

This cross-sectional study was conducted in TU Teaching Hospital. ADS patients were screened by the consultant psychiatrist using the Alcohol Use Disorder Identification Test (AUDIT) questionnaire. The aim of the study and the questions in the questionnaire form were fully explained to the patients. A total of 89 patients scored positive on the AUDIT as having alcohol-related problems and were included in the study. 89 ADS patients and 89 healthy controls both male and female were enrolled as participants. Blood Pressure and other anthropometric parameters were measured while fasting blood samples were analyzed for serum lipid profile. Serum is used for analysis of lipid profile and traditional marker of alcoholism. Total cholesterol was estimated by enzymatic method as described by Allain et al [9]. Serum triglyceride was estimated by Fossati and Prencipe method [10] associated with Trinder reaction [11]. HDL-Cholesterol was estimated by precipitation method, in which chylomichron, LDL-cholesterol and VLDL were precipited and the supernatant fluid containing HDL-cholesterol were estimated by cholesterol method. LDL-cholesterol was calculated using the Friedewald formula [12]. Serum glutamate pyruvate transferase (SGPT), Serum glutamate oxaloacetate transferase (SGOT) and gamma glutamyl transferase (GGT) were estimated by enzymatic method. The test was performed by reagent manufactured by Human, Germany, in the fully automated chemistry analyzer, BT 3000, Italy.

Laboratory standard operation procedures were maintained for all laboratory analysis. Internal quality control sera, both normal and pathological, were also run for each lot of the test, for the validation of the results. SPSS program was used to analyze data, t-test &Spearman's correlation coefficient was used to find correlation.

Results

Mean age of the patients and control subjects was 35.42 ± 5.6 years and 34.53 ± 3.5 years respectively. Range of age was 25-47 years for both groups. The majority of subjects (78%) had begun voluntarily, while 22% claimed to have done so due to peer pressure. The average daily intake of alcohol as stated by the patients was 71.36 gm. The mean duration of drinking was 12.0 years (Range 5-28 years). The frequency of consumption was: daily in 48% patients, 3-5 times a week in 41 % patients. The majority of alcohol dependent patients (72%) consumed alcohol alone while 28% claimed to drink only in company.

Table 1. Comparison of mean age of ADS cases and healthy control

Subject class	Mean±SD	p-value
Cases	35.42 ± 5.6	0.201
Controls	34.53 ± 3.5	

Applied one way ANOVA test, statistically significant at p-value <0.05

Table 2. Comparison of mean of Lipid profile between cases and controls

	Control Mean ± SD	Case Mean ± SD	p-value
Total Cholesterol	3.79±0.74	5.41±0.70	0.001
Triglyceride	1.23±0.60	2.09±0.72	0.001
HDL-C	1.08±0.24	1.66±0.40	0.001
LDL-C	2.15±0.90	2.79±0.81	0.001

Applied one way anova test, statistically significant at p-value <0.05

Table 3. Comparison of mean of different traditional marker and liver enzymes between ADS patients and normal healthy control

	Control Mean±SD	Case Mean±SD	p-value
Gamma-GT	41.80±10.56	181.02±78.16	0.001
MCV	89.77±2.18	97.22±4.6	0.001
SGOT	35.26±14.27	114.35±46.22	0.001
SGPT	26.30±10.6	60.28±13.12	0.001

Applied one way anova test, statistically significant at p-value <0.05

Table 4. Spearman's correlation coefficient of GGT with Lipid profile between cases

	Spearman's rho	p-Values
Total Cholesterol	0.081	0.449
Triglyceride	-0.005	0.964
HDL-C	-0.067	0.528
LDL-C	0.105	0.323

The table 4 shows the Spearman's correlation of GGT with lipid profile between the cases and controls. GGT was not significantly correlated with lipid profile.

Table 5. Spearman's correlation coefficient of SGOT with Lipid profile between cases

	Spearman's rho	p-Values
Total Cholesterol	0.037	0.730
Triglyceride	-0.128	0.228
HDL-Cholesterol	0.128	0.231
LDL-cholesterol	0.020	0.849

The table 5 shows the Spearman's correlation of SGOT with lipid profile between the cases and controls. SGOT was not significantly correlated with lipid profile.

Table 6. Spearman's correlation coefficient of MCV with Lipid profile between cases

	Spearman's rho	p-Values
Total Cholesterol	0.065	0.542
Triglyceride	0.004	0.969
HDL-Cholesterol	0.089	0.407
LDL-cholesterol	0.027	0.804

The table 6 shows the Spearman's correlation of MCV with lipid profile between the cases and controls. MCV was not significantly correlated with lipid profile.

Discussion

The mean total cholesterol levels were found to be higher in cases than controls with a strong statistical significance. Mean TG levels, along with the mean HDL-cholesterol and LDL-cholesterol levels were also elevated in cases when compared to the control samples. Within the group analysis of cases, it revealed borderline high total cholesterol in about half of the cases and very high level of total cholesterol was seen in one-tenth of the patients.

Also, among the cases, majority had elevated triglycerides level. LDL cholesterol was normal in most of the patients. A elevated HDL cholesterol was noted in more than half of the cases. These features are in the line of the notion of cardio protective effect of alcohol consumption by maintaining the level of LDL and HDL with an expense of slightly raised triglyceride level. The present study needs to be seen in the light of earlier studies which appear to confirm the linear relationship between increasing amounts of alcohol use and lipid profile changes known to have protective role for CHD [13]. Alcohol consumption has been found to be associated with increased serum levels of Tg and high density lipoproteins (HDL) [14,15]. The increase in HDL cholesterol has been estimated to account for half of the beneficial effects of alcohol consumption on cardiovascular events.16 Alcohol has narrow therapeutic range and only the moderate drinking has beneficial effects on cardiovascular health [17]. Prolonged excessive drinking causes various structural and functional abnormalities of heart.

Conclusion

In conclusion, this study has demonstrated definitive lipid profile changes in patients of alcohol dependence, with some correlation to the liver dysfunction. Alcohol causes alteration in various parameters of lipid metabolism including those which predispose to CHD. Low to moderate alcohol use over prolonged periods has been linked to have protective influence for development of coronary heart disease (CHD), through increase in high density lipoprotein cholesterol (HDL-C) levels.

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Types of Dyslipidemia in Type 2 DM Patients of Bhubaneswar region

Rajendra Dev Bhatt,[1*] Kamal Lochan[2]

BACKGROUND: A characteristic pattern, termed dyslipidemia, consists of deranged of any single components of lipid profile test. This pattern is most frequently seen in diabetes and may be a preventable risk factor for subsequent cardiovascular disease. This study determined the influence of type 2 diabetes mellitus (T2DM) on lipid profile of diabetic patients reporting in a tertiary hospital in Bhubaneswar, India.

METHODS: 50 confirmed T2DM patients and 50 non-diabetic control subjects were selected for the study. Fasting and 2 hours post prandial blood samples were collected from both study and control patients. Fasting blood sample was analyzed for lipid profile test and serum glucose, and post prandial sample was analyzed for serum glucose only.

RESULTS: Sixty two (62%) of diabetic patients were males whilst thirty eight (38%) were females in this study. The mean plasma glucose levels, Total cholesterol and Triacylglycerol were significantly raised in the diabetics as compared to those in the control subjects. This is substantiated by the fact that the entire lipid fractions are disturbed in diabetics as compared to healthy controls.

CONCLUSION: Thus dyslipidemia was quite common in diabetes and Hypertriglyceridemia was the most common one.

Keywords: Type-2 Diabetes Mellitus, Dyslipidemia, Lipid Profile, Triglyceride, HDL-C, LDL-C, Total Cholesterol.

Introduction

Diabetes mellitus (DM) is a syndrome consisting of metabolic, vascular and neuropathic components that are interrelated. It is defined as group of metabolic disorder that is characterized by hyper-glycemia resulting from defect in insulin secret-ion, insulin action or both. The lack of effective insulin action leads to alteration in carbohydrate, fat and protein metabolism [1]. T2DM is caused by relatively impaired insulin secretion and peripheral insulin resistance [2].

Diabetes is no more an epidemic but it has it has been turned into a global pandemic. Diabetes has been recognized as a health threat worldwide. As per the global projection by international diabetes foundation the number of diabetic patients has risen sharply in recent years. While in 1985, thirty million people had diabetes worldwide; the number rose to one hundred million in 2000, two hundred eighty five million in 2010 and is estimated to be four hundred thirty five million, 7.8% of the adult world population, by 2030 [3].

According to WHO, 70% of current cases of diabetes, occur in developing countries [4]. Among these India has the world's largest population with an estimated 50.8 million people living with diabetes [3]. The international journal of diabetes for developing countries has declared India as the diabetes capital of the world [5].

A characteristic pattern, termed dyslipidemia, consists of increased triglycerides (TAG), Total cholesterol (TC), low density lipoprotein (LDL), and very low density lipoprotein (VLDL) cholesterol and decreased high density lipoprotein (HDL). This pattern is most frequently seen in diabetes and may be a preventable risk factor for subsequent cardiovascular disease.

Patients with T2DM are at greater risk of developing vascular diseases because of lipid changes. Lipid abnormalities and insulin use is critically discussed in diabetics [6]. The most typical lipoprotein pattern reported in diabetes, also known as diabetic dyslipidemia or atherogenic dyslipidemia consists of moderate elevation in TC, TAG and LDL-Cholesterol levels with or without low HDL-Cholesterol levels in blood.

The degree of variations in lipid profile of diabetic patients may not generalized to all region and should be individualized to specific regions as ethnic, hereditary and environmental factors influence lipid profile. Due to increasing cardiovascular problems in T2DM patients [7], this study was conducted to observe the co-

[1]Department of Biochemistry, Hi-Tech Medical College and Hospital, Bhubaneswar, Odisha, India.
Correspondence to: Rajendra Dev Bhatt, Department of Biochemistry, Kathmandu University Hospital-Dhulikhel Hospital, Dhulikhel. E-mail: bhattdev.rajendra@gmail.com

relation of T2DM and types of lipid abnormalities in T2DM. Thus this research aims to evaluate the types of dyslipidemia in T2DM patients of Bhubaneswar region.

Methods

The study was carried out on the patients of medical OPD at Hi-Tech Medical College and Hospital in Bhubaneswar. 50 T2DM patients were recruited after their consent had been sought.

The study targeted T2DM patients, medically diagnosed by American Diabetes Association (ADA) criteria. Randomly selected age and sex matched individuals, with no history of diabetes or any type of illness and not on statins were used as controls.

Patients with type 1 DM, other ailments, metabolic disorders and other causes of hyperlipidemia were not included in this study. Pregnant women, patients on statins for abnormal lipid treatment (both for T2DM and controls) were also excluded.

Venous blood samples were taken from both diabetic and control patients at overnight fasting and 2 hours post prandial state. Investigations carried out were, blood glucose fasting and 2 hrs post prandial blood glucose, and fasting lipid profile including TC, TAG, HDL-C, LDL- C and very low density lipoprotein cholesterol (VLDL-C).

Serum TC was determined by an enzymatic (CHOD/PAP) colorimetric method and TAG was determined by an enzymatic (GPO-PAP) method [8]. HDL-C was estimated by a precipitant method and LDL-C by was estimated by using Friedewald's formula as shown below:

LDL-C = TC - HDL-C – (TAG/5), where TAG/5 is approximately equal to VLDL-C.

Serum glucose was determined by using the glucose oxidase/per oxidase enzymatic method [2].

Dyslipidemia was defined using the National Cholesterol Education Programme – Adult Treatment Panel III (NCEP – ATP III) (National Cholesterol Education Programme, 2002) criteria as shown in Table 1.

Table 1. ATP III Classification of LDL-C, TC, HDL-C and TG (mg/dL).

LDL-Cholesterol	
<100	Optimal
100-129	Near optimal/ above optimal
130-159	Borderline high
160-189	High
>190	Very high
Total Cholesterol	
<200	Desirable
200-239	Borderline high
>240	High
HDL Cholesterol	
<40	Low
>60	High
Triacylglycerol	
<150	Normal
150-199	Borderline high
200-499	High
>500	Very high

Results

In this study 50 diagnosed cases of T2DM were observed as cases and 50 non diabetic were observed as controls. There was significant difference in the fasting blood sugar, post prandial blood sugar level and lipid profile test in control and T2DM patients.

The mean serum TG in our study was 188.9±69.07 mg/dL as compared to 117.56± 23.51 mg/dL of controls. The mean serum total cholesterol was 203.38±48.0 mg/dL as compared to 161.4± 24.55 mg/dL of controls. While HDL-C was 38.22±5.85 mg/dL as compared to 43.46±6.95 mg/dL of controls, which was significantly lower to that of controls as shown in Table 2.

Discussion

We found significant difference ($P<0.0001$) at the Triglyceride level in control and cases where mean ± S.D is 117.56 ±23.51 and 188.9 ±69.07of control and cases respectively. We also found 74% diagnosed diabetics have >150mg/dl, 14% have >250mg/dl and 10% have >300mg/dl of Triglyceride out of 50 cases. And only 6% control have >150mg/dl of Triglyceride.

We also observe significant difference ($p<0.001$) in the Total cholesterol level, where mean±S.D is 161.4±24.55 and 203.38±48.00 of control and cases respectively.

Table 2. Fasting and post prandial serum glucose of type 2 diabetic patients and controls

Parameters	Patients	Controls	P value	T
Fasting serum glucose (mg/dL)	144.62±71.61	85.86±9.33	<0.0001	5.75
Post prandial serum glucose (mg/dL)	209.60±75.49	113.88±15.73	<0.0001	8.78
Triacylglycerol (mg/dL)	188.9±69.07	117.56±23.51	<0.0001	6.91
Total Cholesterol (mg/dL)	203.38±48.0	161.4±24.55	<0.0001	5.51
HDL (mg/dL)	38.22±5.85	43.46±6.95	<0.0001	4.07
LDL (mg/dL)	127.36±45.19	96.32±42.90	<0.0001	4.25
VLDL (mg/dL)	38.90±15.73	23.28±4.70	<0.0001	6.72

We also found 48% of Diabetic have >200mg/dl of Total Cholesterol from 50 cases of diagnosed diabetics, while only 4% control have >200mg/dl of Total cholesterol.

Smith S. and Lall A. M also found mean ±SD of Total cholesterol 299.36+13.46 in their study in Allahabad, India [5].

A significant difference in the HDL and LDL cholesterol level in control and cases. Where mean±S.D is 43.46±6.95 and 38.22±5.86 of HDL and is 96.32±42.90 and 127.36±45.19 of LDL in control and cases respectively. P value of HDL and LDL is <0.0001 which means there is significant difference between control and cases. Among 50 of diabetics 38% have <35mg/dl of HDL and 8% out of 50 controls have <35mg/dl of HDL Cholesterol.

Another study done by Rakesh et al [9] most common pattern was combined dyslipidemia with high LDL and low HDL in both males (22.7%) and females (33%).

In our study, level of TC, TAG, VLDL-C & LDL-C were significantly increased ($p<0.001$) while HDL-C level was significantly decreased ($p<0.01$) in T2DM. These findings are consistent with those of Taha D et al (2002) [10], Howard BV et al (1999) [11], O'Neal DN (1998) [12], Mazanto et al (1993) [13]. Niemeijer et al [14] and they found that increased TAG & decreased HDL-C plasma concentration are common features of dyslipidemia in T2DM. Oki JC in 1995 [15] stated that essentially any dyslipidemic pattern can be present.

In a study by H. Surekha Rani et al [16] an attempt has been made to evaluate the risk factors for coronary heart disease in DM patients. It is observed that, TC, VLDL, LDLs, TGs were high and the levels of HDLs were low compared to controls.

A significant difference was noticed in the VLDL Cholesterol level in control and cases where mean ±S.D is 23.84±4.70 and 38.90±15.73 of control and cases respectively with p value <0.001.

Conclusion

T2DM patients in this study had elevated levels of TAG, TC with slightly elevated levels of LDL-C and reduced levels of HDL-C. This indicates the influence of T2DM on abnormal lipid profile of patients with its associated danger of elevated CVD risk.

Diabetic patients with complication tend to have higher levels of lipid fractions (TAG, T. Chol, LDL and VLDL) and lower level of HDL. This suggests that there appears to be some relation between the geneses of various vascular complications in the presence of lipid abnormality. So it is important to aim at critical control of diabetes mellitus to prevent or at least postpone the onset of various hyperlipidemia related complications.

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Bacteriological and Mycological profile of Chronic Suppurative Otitis Media among patients visiting Dhulikhel Hospital

Vaidya K,[1*]Madhup SK[2], Shrestha BL[2], Gautam A[1], Tuladhar NR[2]

BACKGROUND: Chronic suppurative otitis media (CSOM) is an inflammation of the middle ear and mastoid mucosa with perforation of tympanic membrane. Mainly disease of developing countries like Nepal, CSOM results because of illiteracy, poverty and poor hygiene. Haphazard use of antibiotics and increasing use of newer one has led to persistent change in microbial flora. The aim of this study is to determine the incidence of CSOM and its causative agents.

METHODS: The study included 123 samples from 105 patients attending ENT department of Dhulikhel hospital. Samples were processed in microbiology department for both bacteria and fungi using standard operating protocol. Antibiotic susceptibility testing was performed for all bacterial isolates by Kirby Bauer disc diffusion method and the result were interpreted according to clinical and laboratory standard institute (CLSI) guideline.

RESULTS: Out of 105 patients, 55 were male and 50 female patients. Highest incidence of CSOM was observed between 1-10 years of age group. Of the total 123 samples taken from 105 patients, 106 showed microbial growth. Gram positive bacteria predominated and the most common bacteria isolated were *S. aureus* 54.55% followed by *Proteus spp.* 13.64% and *P. aeruginosa* 12.73%. Among the fungi, the most predominant was *A. fumigatus* 39% followed by *A. niger* 29%, *C. albicans* 26% and *A. flavus* 6%. Gentamycin was the most susceptible antibiotic. *S. aureus* were sensitive to Cloxacillin and Gentamycin, whereas *Proteus spp.* was most sensitive to Ceftriaxone and Norfloxacin. *P. aeruginosa* was 100% sensitive to Amikacin.

CONCLUSION: *S. aureus* was the most predominant organism followed by *Proteus* spp. and the drug of choice was Gentamycin.

Key words: Chronic suppurative otitis media, bacteria, fungi, antibiotic

Introduction

Chronic suppurative otitis media (CSOM) is characterized by a persistant discharge from the middle ear through a tympanic membrane perforation [1].The disease is classified into tubotympanic and atticoantral depending upon where the disease affects [2].

It is an important cause of preventable hearing loss, particularly in the developing world [1]. Annually it affects between 65 to 330 million individuals and cause 28000 deaths per annum [1]. In Nepalese context prevalence of CSOM is 5% which is higher than other countries and more than 55% of these cases occur in school going children, most of them belonging to the lower socio-economic class [3].

Most commonly isolated aerobic bacteria in CSOM are *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes, Proteus mirabilis, Klebsiella spp* [4]. Fungal infection of the middle ear and external auditory meatus are common as fungi thrive well in moist pus and the mostly isolated fungi are *Candida* species and *Aspergillus* species. But the type of organism isolated varies between the geographical areas and other factors [5].

It is nowadays rare for an otologist to see ears with discharge that have not already had the bacterial flora modified by antibiotic therapy since most patients attend the hospital very late when treatment becomes a problem and cultures are frequently sterile. This may be because of microbial resistance to these antibiotics thereby suggesting their failure leading to continuation of purulent discharge in the discharging ear [6]. Hence, providing pattern of modern day isolates and their sensitivity toward antibiotics was the centre of this study.

Methods

Cross sectional descriptive analytical study was carried out at the department of microbiology in Dhulikhel hospital, Kathmandu University Hospital from April 2011 to March 2012. One

[1]Department of Microbiology, St. Xavier's College, Maitighar, Kathmandu, Nepal.[2]Dhulikhel Hospital, Dhulikhel, Kavre, Nepal

Correspondence to: Ms. Krista Vaidya. E-mail: krissvsn@gmail.com

hundred and five clinically diagnosed CSOM patients, having ear discharge for more than 3 months with unilateral or bilateral disease attending out-patient department of ENT, were included in this study. An otolaryngologist diagnosed and evaluated CSOM by otoscopic examination. Then using sterile cotton swab, ear discharge was collected through perforation in the tympanic membrane via sterile aural speculum to avoid contamination from the skin of the auditory canal. In case of bilateral infection, samples were taken from both ears. So, in total 123 middle ear samples were collected from the total 105 CSOM patients. Samples were then immediately transported to Microbiology department.

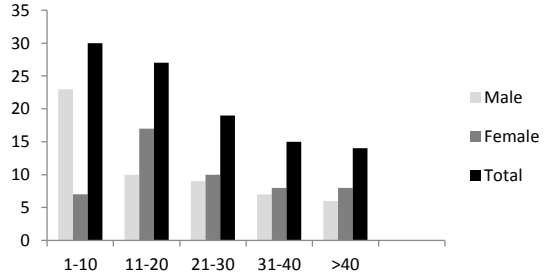
Each patient requested for culture was directly interviewed for his or her clinical history during sample collection. The preformed performa was filled documenting age, sex, and clinical information including side of ear affected, and types of CSOM.

Samples were inoculated into sheep blood agar (SBA), MacConkey agar (MA) and Chocolate agar (CA) for isolation of aerobic bacteria and incubated aerobically at 37°c for 24 hours. For isolation of fungi, samples were inoculated into two sabouraud dextrose agar (SDA); one incubated at 25°c and other incubated at 37°c for upto 7 days. Antibiotic susceptibility testing of bacterial isolates was performed on muller hinton agar (MHA) by Kirby Bauer disc diffusion method using CLSI guideline [7]. Results obtained were analysed by SPSS version 16.

Results

Out of 105 cases studied, 78(74%) had tubotympanic type of CSOM and 27(26%) had atticoantral type. Unilateral infection was seen in 87 (82.85%) patients and bilateral in 18 (17.15%). Of the cases studied, 55 (52.38%) were male and 50 (47.61%) female. Similarly, higher incidence of CSOM was seen in 1-10yrs of age group and it decreases as the age increases. Figure 1 shows the age and gender wise distribution of CSOM. Among the 105 patients, no growth was seen in 14 patients. Of the 91 patients showing microbial growth, 46 were male and 45 female. Age-wise high microbial growth was observed in 11-20 years (24) and 1-10 years (23) followed by 21-30 years (16) and age group 31-40 and > 40 showed equal number of growth i.e. 14.

Fig 1. Age and gender wise distribution of CSOM



Of the 123 samples collected from 105 patients, 106 showed microbial growth and 17 showed no growth. Of the total growth, 64 were pure bacterial isolates, 9 pure fungal and 33 mixed types. Total 141 microorganisms were isolated in this study, gram positive bacteria predominated; *Staphylococcus aureus* being the most predominated one followed by *Proteus spp* and *Pseudomonas aeruginosa* respectively. In case of fungal isolates, *Aspergillus* species predominated of which *A. fumigatus* prevailed (Table 2).

Out of 63-gram positive bacteria isolated, 59 (93.65%) were susceptible toward cloxacillin, 58 (92.06%) toward gemtamycin and 56 (88.89%) toward chloramphenicol. 59 (93.65%) gram-positive bacteria showed resistance against Penicillin.

Antibiotic susceptibility testing done for gram-negative bacteria except *Pseudomonas aeruginosa* showed higher susceptibility toward gentamycin (91.9%), ceftriaxone (87.9%), norfloxacin (84.85%) and ciprofloxacin (81.8%).

Staphylococcus aureus the most predominant organisms isolated in this study was found to be most sensitive toward cloxacillin and gentamycin whereas most of the isolates were resistant to penicillin. Out of 60, Staphylococcus aureus isolated, 4 (6.67%) were found to be methicillin resistant (Table 3). Similarly, *Proteus* species were 100% sensitive to ceftriaxone and norfloxacin (Table 3). The antibiotic susceptibility testing for *Pseudomonas aeruginosa* showed 100% sensitivity toward amikacin (Table 5).

Table 1. Bacteriological profile in CSOM

Type of bacteria	Organism	No. of isolates (n=110)	%
Gram positive	*Staphylococcus aureus*	60	54.54
	Enterococcus spp.	1	0.91
	Streptococcus pneumoniae	2	1.82
Gram negative	*Proteus mirabilis*	6	5.45
	Proteus vulgaris	9	8.18
	Pseudomonas aeruginosae	14	12.73
	Klebsiella oxytoca	4	3.64
	Klebsiella pneumoniae	2	1.82
	Escherichia coli	5	4.54
	Acinetobacter spp.	2	1.82
	*Enterobacter*spp	3	2.73
	Citrobacter spp	2	1.82

Table 2. Fungal profile in CSOM

Organism	No. of isolates (n=31)	%
A. fumigatus	12	38.71
A. niger	9	29.03
A. flavus	2	6.45
C. albicans	8	25.81

Table 3. Antibiotic susceptibility pattern of *Staphyloccous aureus*

Antibiotics (µg)	Sensitive (%)	Resistant (%)	Intermediate (%)
Cloxacillin (10)	93.33	6.67	0
Penicillin (10)	1.67	98.33	0
Cefazolin (30)	73.33	16.67	10
Erythromycin (15)	50	30	20
Gentamycin (10)	91.67	8.33	0
Ciprofloxacin (5)	51.67	33.33	15
Chloramphenicol (30)	88.33	3.33	8.33

Table 4. Antibiotic susceptibility pattern of *Proteus spp*

Antibiotics (µg)	*Proteus mirabilis* (n=9)			*Proteus vulgaris* (n-6)		
	Sensitive (%)	Resistant (%)	Intermediate (%)	Sensitive (%)	Resistant (%)	Intermediate (%)
Gentamycin (10)	77.78	22.22		100		
Ciprofloxacin (5)	100			66.67	33.33	
Cephalexin (30)		88.89	11.11	33.33	50	16.67
Ceftriaxone (30)	100			100		
Norfloxacin (10)	100			100		
Tetracycline (30)		100		66.67	33.33	
Amoxycyllin (10)	88.89	11.11		50	50	

Table 5. Antibiotic susceptibility pattern of *Pseudomonas aeruginosa*

Antibiotics (µg)	Sensitive (%)	Resistant (%)	Intermediate (%)
Amikacin (30)	100	0	0
Ceftazidime (30)	85.71	14.29	0
Carbenicillin (100)	78.57	21.43	0
Piperacillin (100)	28.57	71.43	0
Ciprofloxacin (5)	92.86	0	7.14
Gentamycin (10)	92.86	7.14	0

Discussion

In this study male predominance was higher (52.38%) than female. It is in accordance to other studies [8-10] but is in contrast to study done by Loy et al and Mansoor et al [11, 12]. Age group 1-10 years had the higher prevalence of CSOM 28.57%. Study done by Shrestha et. Al. and Jha et. Al. also found the similar result [13, 14]. High prevalence rate in children may be due to multiple reasons as young children and infants may have low resistance and also because of relatively short Eustachian tube. Due to short Eustachian tube, infected material from the nose, adenoids and sinuses passes more readily along the Eustachian tube to the tympanic cavity, particularly during coughing, sneezing, vomiting, and forced feeding commonly practiced in our environment with the child's nose blocked, while being held head down and half prone [15]. It may be also attributed to the fact that they are more prone to upper respiratory tract infections. Furthermore, cold weather predisposes children to upper respiratory tract infection. Poor hygiene and unorthodox approach to treatment like use of unconventional ear drops and concoctions such as oil and honey into the middle ear may initiate the proliferation of opportunistic pathogens leading to blockage of Eustachian tube [16].

In the present study *Staphylococcus aureus* was found to be the most predominant organism 54.54% followed by *Proteus spp* 13.61% and *Pseudomonas aeruginosa* 12.73%. Higher prevalence of *S. aureus* in this study also resembled very much with Park et al having 54% of *S. aureus* [17]. The result is also in harmony with other studies [9, 13, 18-20]. In contrast, Gul et al and Iqbal et al isolated higher proportion of *P. aeruginosa* [21, 22].The reason behind *S. aureus* to be most prevalent organism might be because it is an opportunistic pathogens and a normal flora of skin, but when it gains entrance into the human body it causes infection to tissues and mucous membrane [23]. In different previous studies [16, 24, 25], anaerobic bacteria were also isolated but we have not included anaerobic bacteria in this study.

Thirty one (21.98%) fungal isolates were obtained in the present study which was similar to the previous study carried out by Nia et al in which 24.57% of fungi were isolated [20].It has been postulated that the prolonged use of topical broad spectrum antibiotics leads to suppression of bacterial flora and subsequent emergence of opportunistic fungal flora in the areas of oral cavity, gastrointestinal tract and vaginal tract. Likewise in case of the middle ear, fungal infection supervenes because of prolonged use of topical antibiotics. It happens because of settling of fungal elements like spores from external environment in the moist and alkaline medium of middle ear discharge and debris. This finally leads to the development of mycotic otitis media causing intractable otorrhoea [26]. Of the fungal isolates, *Aspergillus spp*. predominated which was similar with the study conducted by Shrestha et al and Loy et al but in contrast, Parveen and Rao found *Candida spp*. to be the most common fungal isolates whereas only *Candida spp*. were isolated in the study done by Nwabuisi and Ologe [11, 13, 20, 27].

In this study almost all bacterial isolates were found to be sensitive toward gentamycin. Study done in Bir hospital and Om hospital, Nepal also found gentamycin as one of the effective antibiotic [14, 28]. In the present study *S. aureus* the most predominated organism had high sensitivity toward cloxacillin and gentamycin. The result is similar to the restrospective study carried out in Bharatpur, Nepal [29]. Of the *S. aureus* isolated, 4(6.67%) were methicillin resistant which was similar with the result of Iqbal et al [22]. Isolation of MRSA could be community acquired infection as all the patients included were out patients [22].

Conclusion

Staphylococcus aureus was the most common isolates and was most sensitive to Cloxacillin followed by Gentamycin and Chloramphenicol. For overall bacterial isolates Gentamycin was found to be the most effective drug. *Aspergillus spp*. was the mostly isolated fungal isolates of which *A. fumigatus* predominated.

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A cross-sectional study of lung functions in traffic police personnel at work in Kathmandu Valley, Nepal

Hari Sunder Shrestha,[1*]Ojashwi Nepal,[2]Kishor Khanal,[3]Bhoopinder Kumar Kapoor[2]

BACKGROUND: The present study was aimed to assess pulmonary functions in the traffic police personnel (TPP) posted on traffic duty in Kathmandu valley, Nepal.

METHODS: The study group consisted of 17 females and 89 males, constituting 16% and 84% of the total police personnel studied, respectively. In the control group of 25 individuals, 16% (n=4) were female and 84% (n=21) were male. Portable desktop spirometer was used for the pulmonary function test (PFT) measurements.

RESULTS: It is seen that in females as compared to males, PFT parameters show a significant decrease. One-way ANOVA conducted to compare the effect of duration of air pollution exposure showed that there is a significant variation in PFT parameters among the groups. The exposure duration has significant effect on the PFT parameters.

CONCLUSION: Greater the officers are engaged in traffic duty for years, greater is the decrement in their lung functions test.

Key words: Traffic Police Pulmonary Spirometer Air Function

Introduction

During the last few decades, air pollution of the urban atmosphere has received much attention and several studies have linked it with serious health risks especially respiratory diseases [1] According to the World Health Organization (WHO), air pollution is responsible for increase in out-patient visits, hospital admissions, and mortality due to respiratory and cardiovascular diseases [2] Traffic police personnel (TPP) working in outdoor urban environments are occupationally exposed to pollutants generated by engine combustion. A study carried out in Rome on personnel exposure to benzene found that traffic police personnel's exposures were consistently higher than those of personnel who did office work [3]. Another study conducted in Turkey showed that the TPP are the population group under risk due to the inhalation of carbon monoxide (CO) rich air while on duty at the crowded cross-sections of the city [4]. Deteriorating quality of air is a growing concern in most countries, including Nepal. Vehicular emission is the major cause of outdoor air pollution in Kathmandu Valley [5]. Bowel shape topography, temperate climate, and tropical monsoon further worsen the effect. For these reasons, the valley is classified as a High Air Pollution Potential Zone (HAPPZ) [6]. Shrestha and Malla in 1996 estimated air pollution load in Kathmandu valley by different sectors, based on the use of energy and found transport sector to have the largest contribution in total emissions followed by the household, industrial, and commercial sectors [7].

Urban air quality management strategy in Asia reported that peak particulate matter of diameter 10 microns or less (PM10) concentration is 800 microgram per cubic meter (μg/m^3) in Kathmandu [8].Annual PM10 in high traffic area was found to be 261.4±28.5 μg/m^3 [9]. Kathmandu's ambient air quality usually crosses international guidelines by two to three folds [10]. The population residing in the valley is risk group prone to develop air pollution related respiratory diseases and the most vulnerable groups include traffic police personnel, street vendors, shopkeepers, etc. Kandel reported that 6.4% TPP studied by her in Kathmandu showed lower FEV1/FVC ratio as compared to normal [6]. Acharya concluded that TPP in Kathmandu were more exposed to particulate matter and most of them complained of fatigue, back/neck problems, arthritis, dryness of nose, forgetfulness, headache, irritation, indigestion, and stress [11] Recently, Anobha et al have demonstrated that air pollution in Kathmandu valley has substantial health impacts [12]. Shakya found that most of the personnel who were working in Kathmandu valley suffered from problems of the respiratory system and nervous system [13]. Considering these findings, the traffic police personnel, who are exposed to air pollution, are most likely to have impaired pulmonary function. Therefore,

[1]Department of Physiology, KIST Medical College, Imadole, Lalitpur, Nepal. [2]Department of Physiology, [3]Department of Community Medicine, Kathmandu University School of Medical Sciences, Dhulikhel, Kavre, Nepal

Correspondence to: Ojashwi Nepal, Department of Physiology, Kathmandu University School of Medical Sciences, Dhulikhel, Kavre, Nepal. E-mail: ojan2nep@gmail.com Phone: +977-9851087866

the present study was planned to assess pulmonary functions in the traffic police personnel posted on traffic duty in Kathmandu valley, Nepal.

Methods

This study was conducted in Kathmandu valley. The present comparative study includes randomly selected 106 TPP from those engaged in traffic control with the exposure to automobile exhausts. There are currently around 1100 traffic police personnel stationed at 33 units in the Kathmandu valley. Out of them, this study include 9% of the total number i.e. 106 traffic police personnel. This epidemiological study includes both male and female. Out of total 106 traffic police personnel 89 were male and17 subjects were female. The "Control" includes 25 individuals involving all those in non-traffic job in Kathmandu valley. The study group consisted of 17 females and 89 males, constituting 16% and 84% of the total police personnel studied, respectively. In the control group of 25 individuals, 16% (n=4) were female and 84% (n=21) were male. The ethical clearance was obtained from institutional review committee of Kathmandu University School of Medical Sciences (KUSMS) and was in accordance with the Helsinki Declaration of 1975, as revised in 1983.

The study was conducted over a period of 6 months. Health camps were organized for the medical examination of traffic police during the study period. The first camp was conducted on 26th November 2012. Health examination and questionnaire survey was carried out in camps on Saturdays, and approximately 15 subjects were examined on each health camp.

The work experience of more than or equal to two years was required as inclusion criterion for the TPP to be included in this study. Primary data was collected from the participants of the study by direct interview based on the study questionnaire. The questionnaire survey, an occupational health questionnaire was designed based on the modified ATS-DLD-78-A (The American Thoracic Society, Division of Lung Diseases) [14]. On the day of their medical examination, written consent was taken from each of the subjects for the questionnaire survey and the medical examination. The subjects were explained about the actual procedure and purpose of the study before they were put to test. The participants were asked not to have large meal few hours before the test and to wear loose clothes for the test. The check-up included anthropometric measurements like height in centimeter (cm), weight in Kilogram (Kg), calculation of Body Mass Index-BMI (Kg/m^2) and measurement of blood pressure. Portable desktop spirometer, MIR Spirolab III was used for the PFT measurements. The procedure was conducted in the morning hours and ensured that the subject was not exposed to air pollution at least for 10 hours before the test. PFT was performed in all subjects in the standing position.

The data obtained from the questionnaire and medical examinations were analyzed using the software Microsoft Excel (MS-Excel) and Statistical Package for Social Sciences (SPSS) version 16. The data from the research were processed for mean, standard deviation, independent sample t-test, and one-way ANOVA. Descriptive statistics of the parameters were presented as means and standard deviations. Values for probability less than 0.05 ($p<0.05$) at 95% confidence interval were considered statistically significant.

Results

It was found that in majority of cases, the age of personnel interviewed were within the range of 26-30 years. The mean age was found to be 30.43±6.12 years. 37% (n=39) of the respondents were educated up to School Leaving Certificate, 46% (n=49) up to Intermediate level, while 17% (n=18) had Bachelor's degree. The majority of the study group were Constable 60% (n=64) followed by Head Constable (n=19), Assistant Sub-Inspector (n=10), Sub-Inspector (n=10), and Inspector (n=3). Three percentage (n=3) of the respondents were involved in the service for more than 15 years, 13% (n=14) were involved in their service for 11-15 years, 52% (n=55) for 6-10 years, and 32% (n=34) for 2-5 years. Maximum number of respondent (17%) was posted at Singha Durbar while only 4% worked at Kalimati. However, it was intimated that the duty station was usually shifted every six months. Out of the total respondents 6.6% (n=7) consumed alcohol while 15.1% (n=16) chewed tobacco regularly.

Regarding personal health care by respondents such as use of PPE (Personal Protective Equipment) example mask, involvement in yoga/exercise, and health check-up, most of the respondents (79.2%) were using masks regularly

while 20.8% used nothing. 32.1% of the participants were involved in physical activities like yoga/exercise and 18.9% of the respondents visited medical personnel for their health checkup on yearly basis.

It was found that there was no significant difference between the two groups for age [study group, 30.43±6.12 years Vs control group, 31.60±7.98 years, p=0.499], weight [study group, 66.42±10.10 kg Vs control group, 64.76±15.80kg, p=0.514], BMI [study group, 22.55±3.26 kg/m^2 Vs control group, 23.18±4.76 kg/m^2, p=0.428], and body surface area (BSA) [study group, 1.77±0.14 m^2 Vs control group, 1.72±0.20 m^2, p=0.102]. However, there was no consistency in the height of individuals in both groups [study group, 171.62±6.89 cm Vs control group, 166.80±9.06 cm, p=0.018].

Table 1 shows the results of PFT obtained in traffic police personnel along with the comparison of PFT parameters between TPP (study group) and control group. There is significant decrease in parameters like forced vital capacity (FVC), forced vital capacity expressed as body surface area (FVC_BSA), forced expiratory volume in 1 second (FEV1) forced expiratory volume in 1 second expressed as body surface area (FEV1_BSA), peak expiratory flow rate (PEFR), peak expiratory flow rate expressed as body surface area (PEFR_BSA), forced expiratory flow at 25% of volume as a percentage of FVC (FEF25%), maximum voluntary ventilation (MVV), and maximum voluntary ventilation expressed as body surface area (MVV_BSA) at p value <0.01 level, a significant decrease in forced expiratory flow at 25% of volume as a percentage of FVC expressed as body surface area (FEF25%_BSA) at p value <0.05 level while no significant gender difference in FEV1/FVC%, forced expiratory flow at 25-75% of volume as a percentage of FVC (FEF25%-75%) and forced expiratory flow at 25-75% of volume as a percentage of FVC expressed as body surface area (FEF25%-75%_BSA) was found.

Table 2 shows that within the study group, comparing the PFT parameters between personnel on traffic duty for less than eight years and those for eight or more years, a significant decreases in parameters like FVC, FEV1, FEF25%-75%, forced expiratory flow at 50% of volume as a percentage of FVC (FEF50%), forced expiratory flow at 75% of volume as a percentage of FVC (FEF75%), and MVV (all expressed per square meter body surface area) were found in personnel on traffic duty for more than eight years.

A one-way ANOVA as shown in Table 3 was conducted to compare the effect of duration of air pollution exposure. For this purpose, the study sample personnel were divided into four groups, based on the duration of their traffic duty in years: Group I 2-5 years, Group II 6-10 years, Group III 11-15 years, and Group IV >15 years. There is a significant variation in PFT parameters like FVC_BSA, FEV1_BSA, FEF75%, and FEF75%_BSA among the groups.

Table 1. Comparison of PFT parameters between TPP (study group) and control group

Parameters	Study group (n=106)	Control group(n=25)	p value
FVC (L)	4.01±0.66	4.09±0.49	0.496
FVC_BSA	2.25±0.33	2.39±0.32	0.058
FEV_1 (L)	3.35±0.57	3.41±0.57	0.635
FEV_1_BSA	1.89±0.30	2.00±0.35	0.114
FEV_1/FVC%	83.88±6.66	83.27±7.25	0.690
PEFR (L/s)	8.28±1.95	8.75±1.38	0.172
PEFR_BSA	4.66±1.04	5.11±0.75	0.042*
$FEF_{25\%-75\%}$(L/s)	3.80±1.087	4.42±0.98	0.011*
$FEF_{25\%-75\%}$_BSA	2.15±0.63	2.59±0.59	0.002*
$FEF_{25\%}$(L/s)	6.70±1.87	7.48±1.12-	0.009*
$FEF_{25\%}$_BSA	3.76±1.00	4.38±0.66	0.000**
$FEF_{50\%}$(L/s)	4.22±1.22	4.71±1.07	0.067
$FEF_{50\%}$_BSA	2.38±0.70	2.76±0.63	0.016*
$FEF_{75\%}$(L/s)	1.75±0.70	2.20±0.99	0.039*
$FEF_{75\%}$_BSA	0.99±0.43	1.28±0.56	0.006*
MVV (L/min)	130.92±27.08	129.81±35.55	0.885
MVV_BSA	73.80±14.87	76.32±21.48	0.583

Values are Mean±SD; n: number of participants

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

There is significant effect of exposure duration on FVC (L/m^2) [F=4.299, p =0.007], on FEV1 (L/m^2) [F=4.232, p =0.007], and on FEF75% (L/s/m^2) [F=4.400, p =0.006], in the aforementioned four groups.

Further, Post hoc comparisons using the LSD test indicated that the mean FVC (L/m^2) of Group I TPP to whom duration of exposure was 2 to 5 years (2.28±0.34 L/m^2) was significantly different from the mean FVC (L/m^2) of Group III TPP to whom duration of exposure was 11 years to 15 years (2.07±0.28 L/m^2) and Group IV TPP to whom exposure duration was more than 15 years (1.79±0.13 L/m^2).

Post hoc comparisons using the LSD test indicated that the mean FEV1 (L/m^2) of Group I TPP (1.96±0.35 L/m^2) was significantly different from the mean FEV1 (L/m^2) of Group III TPP (1.71±0.20 L/m^2) and Group IV TPP (1.50±0.16 L/m^2).

Table 2 Comparison of PFT parameters between TPP exposed to less than eight years and TPP exposed to eight or more years.

Parameters	Duration of exposure		p value
	<8 years (n=65)	≥8 years (n=41)	
FVC_BSA	2.34±0.32	2.11±0.29	0.000**
FEV_1_BSA	1.97±0.32	1.75±0.22	0.000**
FEV_1/FVC %	84.12±7.41	83.49±5.33	0.639
PEFR_BSA	4.67±1.07	4.64±0.99	0.877
$FEF_{25\%-75\%}$_BSA	2.26±0.69	1.96±0.45	0.009*
$FEF_{25\%}$_BSA	3.78±1.01	3.73±0.98	0.812
$FEF_{50\%}$_BSA	2.48±0.78	2.22±0.52	0.038*
$FEF_{75\%}$_BSA	1.09±0.49	0.84±0.25	0.001**
MVV_BSA	76.66±14.86	69.28±13.89	0.012*

Values are Mean±SD; n: number of participants
** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

Table 3. One-way ANOVA for comparison of pulmonary function tests of TPP according to duration of exposure

Parameters	2-5 years (n=34)	6-10 years (n=55)	11-15 years (n=14)	>15 years (n=3)	F value	p value
FVC (L)	3.96±0.75	4.12±0.60	3.78±0.62	3.46±0.61	1.897	0.135
FVC (L/m^2)	2.28±0.34	2.30±0.31	2.07±0.28	1.79±0.13	4.299	0.007**
FEV_1 (L)	3.38±0.68	3.42±0.52	3.12±0.45	2.89±0.60	1.688	0.174
FEV_1 (L/m^2)	1.96±0.35	1.91±2.27	1.71±0.20	1.50±0.16	4.232	0.007**
FEV_1 %	85.64±6.95	83.03±6.64	83±6.24	83.45±3.52	1.182	0.320
PEFR (L/s)	8.04±2.07	8.56±1.91	7.65±1.93	8.98±0.15	1.156	0.331
PEFR (L/s/m^2)	4.63±1.01	4.78±1.07	4.20±1.02	4.71±0.43	1.185	0.319
$FEF_{25\%-75\%}$ (L/s)	3.97±1.21	3.80±1.04	3.57±0.90	3.11±0.90	0.902	0.443
$FEF_{25\%-75\%}$ (L/s/m^2)	2.32±0.74	2.12±0.57	1.96±0.49	1.60±0.32	2.065	0.110
$FEF_{25\%}$ (L/s)	6.44±1.63	6.93±2.07	6.33±1.71	7.06±1.16	0.701	0.553
$FEF_{25\%}$ (L/s/m^2)	3.72±0.85	3.87±1.12	3.48±0.92	3.68±0.41	0.575	0.633
$FEF_{50\%}$ (L/s)	4.35±1.34	4.23±1.21	3.97±1.09	3.56±0.63	0.603	0.614
$FEF_{50\%}$ (L/s/m^2)	2.53±0.80	2.36±0.66	2.18±0.60	1.85±0.14	1.507	0.217
$FEF_{75\%}$ (L/s)	2.01±0.94	1.69±0.52	1.47±0.44	1.20±0.66	3.159	0.028*
$FEF_{75\%}$ (L/s/m^2)	1.18±0.59	0.94±0.29	0.81±0.24	0.61±0.28	4.400	0.006**
MVV (L/min)	129.32±29.56	134.64±26.0	122.91±26.45	118±12.63	1.023	0.389
MVV (L/min/m^2)	74.86±15.14	75.49±15.07	67.20±12.62	61.77±6.57	1.916	0.132

Values are Mean±SD; n: number of participants
** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

In addition, the mean FEV1 (L/m^2) of Group II TPP (1.91±2.27 L/m^2) was significantly different from the mean FVC (L/m^2) of Group III TPP (1.71±0.20 L/m^2) and Group IV TPP (1.50±0.16 L/m^2). The mean FEV1 (L/m^2) of Group I TPP (1.96±0.35 L/m^2) did not significantly differ from the mean FEV1 (L/m^2) of Group II TPP (1.91±2.27 L/m^2). Also, the mean FEV1 (L/m^2) of Group III TPP (1.71±0.20 L/m^2) did not significantly differ from the mean FEV1 (L/m^2) of Group IV TPP (1.50±0.16 L/m^2).

Post hoc comparisons using the LSD test indicated that the mean FEF75% ($L/s/m^2$) of Group I TPP (1.18±0.59 $L/s/m^2$) was significantly different from the mean FEF75% ($L/s/m^2$) of Group II TPP (0.94±0.29 $L/s/m^2$), Group III TPP (0.81±0.24 $L/s/m^2$), and Group IV TPP (0.61±0.28 $L/s/m^2$).

The mean FEF75% ($L/s/m^2$) of Group II TPP (0.94±0.29 $L/s/m^2$) did not significantly differ from the mean FEF75% ($L/s/m^2$) of Group III TPP (0.81±0.24 $L/s/m^2$) and Group IV TPP (0.61±0.28 $L/s/m^2$). Also, the mean FEF75% ($L/s/m^2$) of Group III TPP (0.81±0.24 $L/s/m^2$) did not significantly differ from the mean FEF75% ($L/s/m^2$) of Group IV TPP (0.61±0.28 $L/s/m^2$).

The prevalence of various respiratory symptoms on the basis of the analysis of the questionnaire, cough was present in 41 volunteers, phlegm in 34 individuals, breathlessness in 27 of them, and chest illness in 7 subjects among 106 cases studied.

Discussion

The PFT parameters of most of the TPP were within the normal range. But when they were compared with the control group, it was found that they were significantly inferior to those of controls. The Spiro metric parameters PEFR, FEF25%-75%, FEF25%, FEF50%, and FEF75% all expressed per square meter body surface area were significantly reduced in TPP than in controls.

FVC per square meter body surface area statistically did not show significant difference between traffic police personnel and controls. However, there was slight decrease in the value for the study group. Volume of air expired in the 1st second of the test expressed as BSA was also statistically insignificant between both the groups. However, there was slight decrease in the value for the study group. These findings contradict those of Wonsurakiat et al., Singh et al., Pal et al., and Sharat et al. who found consistent difference in FVC and FEV1 between TPP exposed to vehicular emission and those not exposed [15-18].

The FEV1/FVC ratio is a better indicator of the condition of the bronchial musculatures. FEV1 percentage of FVC in both the groups was found statistically insignificant.

Peak expiratory flow rate (PEFR), which is also termed maximal expiratory flow, occurs shortly after the onset of expiration. The PEFR, more than the other measures, is dependent on participant's effort, thus signifying the capacity of expiratory muscles. In this study, the significant decrease in PEFR value in case of TPP indicates that there was some obstruction during expiration. This is similar to the finding of other investigators [19,20].

FEF25%-75% indicates flow rates in small airways i.e. those with internal diameters of less than two millimeter. Decrease in FEF25%-75% suggests greater involvement of small airways. The average forced expiratory flow rate over the middle 50% of the FVC (FEF25%-75%) which explains the patency of smaller airways was found to be significantly different between both the groups with higher reduction in the value in case of exposed traffic police personnel. The FEF25%-75% being relatively sensitive index of airflow obstruction and may be abnormal when the FEV1/FVC ratio is still preserved. It is affected by the airway resistance during forced expiration.

Increased average levels of total suspended particles (TSP) over a 4-days period are significantly associated with decrements in FVC, FEV1, and FEF25%-75% [21].

In the present study FEF25% expressed per square meter body surface area of TPP (which suggests conditions of larger airways) was also significantly decreased. Reduction in both FEF25%-75% and FEF25% in TPP suggests that the airways in generalare narrowed preventing the free flow of air during respiration. Similar results were reported by Pal et al. [17]. FEF50% and FEF75% both expressed per square meter body surface area were also found to be significantly reduced in TPP as compared to control groups.

Maximum voluntary ventilation (MVV) is dependent on the muscular development of the individual. The subject is instructed to breathe as hard and fast as possible for 10 to 15 seconds. The result is extrapolated to 60 seconds and reported in liters per minute. A low MVV can occur in obstructive disease, in restrictive disease, in neuromuscular disease, in heart disease, in a patient who does not try hard enough or does not understand how to perform the test, or in a frail patient. Thus, this test is very nonspecific, and yet it correlates well with a subject's exercise capacity and with the complaint of dyspnea. In this study, MVV though statistically not significant, was found to be decreased in TPP.

Comparison of PFT parameters between personnel on traffic duty for eight or more years and those for less than eight years showed significant decrease in parameters like FVC, FEV1, FEF25%-75%, FEF50%, FEF75%, and MVV (all expressed per square meter body surface area) in greater exposed personnel. This finding was similar with the study of Gupta et al. [18].

Comparison of PFT parameters (expressed per m^2 BSA) among TPP in different groups, representing duration for which they have been on traffic duty revealed significant decreases, the magnitude of decrease in many PFT parameters being broadly correlated with the duration of traffic duty. This suggests that increase in the duration of traffic duty (in years) has increasingly harmful effect on lung function in traffic police personnel.

No significant differences were found in FVC and FEV1 between Group I and Group II TPP. FEF75%in Group I TPP significantly differs from that in other groups of TPP with the successive reduction in their values in Group II and III. In this study pulmonary function tests like FVC (L/m^2) and FEV1 (L/m^2) in Group II TPP were not significantly different from those in Group I, but FEF75% (L/s) and FEF75% ($L/s/m^2$) were. The most sensitive measure in our study was found to be FEF75%.

Although TPP in Group IV showed the same trend, a large sample is needed for definite quantitative analysis of PFT in TPP exposed for more than 15 years. Their PFT parameters were not subjected to statistical comparisons, since the number of TPP in this group was small (only 3).

These findings suggest that the exposure duration has significant effect on the PFT parameters; greater the officers are engaged in traffic duty for years, greater is the decrement in their lung functions tests.

There is a normal gender dependent physiological variation in Spiro metric test values. Males usually show higher values than the females [22]. In this study, comparison of PFT parameters between male TPP and female TPP showed lower values of FVC, FEV1, PEFR, FEF25%, and MVV (all expressed per square meter BSA) in the female TPP than in the male TPP.

The number of females in the study was 17. Only 5 of them were on traffic police duty for more than 5 years. Thus the great majority of them were involved in traffic control duty only for a short period of time (2-5 years). And yet, the female TPP showed a far greater reduction in PFT parameters than their male colleagues.

Detailed work on a larger number of women TPP involved in traffic duty over a prolonged period of time is required to further explain and elucidate the present findings.

The questionnaire survey carried out among TPP and the controls revealed that many of the participants had short term respiratory effects such as cough, phlegm, and breathlessness confirming the findings of other studies [6, 11, 13, 18].

Traffic police personnel in Kathmandu have been provided with masks to wear while on duty. Many of them reported that they found it inconvenient to wear, as on many an occasion, it interfered with their duties (like need to whistle!). However, the present study did not study the effect of wearing or not wearing a mask on the PFT.

Conclusion

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Cartilaginous Choristoma in Tonsil : A Rare entity

Sujan Sharma,[1*] Ramesh Makaju,[1] Bikash Shrestha[2]

Choristoma is a tumor like mass consisting of tissues foreign to the site at which they are located. We report a 39-year-old female who presented to our out-patient department with history of pain and burning sensation in the throat. On examination of the tonsils, bilateral keratosis was present. Histological examination demonstrated the unexpected presence of mature hyaline cartilage surrounded by lymphoid follicles.

Keywords: Tonsil; Cartilaginous Choristoma

Introduction

Choristoma is an island of normal tissue in an abnormal location Cartilaginous choristoma was first described by Berry in 1890 [1]. The age of diagnosis varies greatly from 10 to 80 years. Choristoma in the head and neck region was reported in the pharynx, hypopharynx, oral mucosa and middle ear [2]. Here we report a case of 39-year-old female who was clinically diagnosed as tonsillar keratosis. On histopathological examination mature hyaline cartilage were found surrounded by lymphoid follicles.

Case Report:

A 39-year-old female who presented to our Otolaryngology out patient department with history of pain and burning sensation in the throat right side was more than the left .The remainder of the head and neck was free of mass lesions and other significant findings. On examination of the tonsils, bilateral keratosis was present. Patient was admitted in the Otolaryngology ward and was managed surgically as there was no improvement despite all conservative measures. Operative findings was grade I enlarged bilateral tonsils with keratosis in bilateral tonsillar crypts. Tonsillectomy was performed and the specimen was sent for histopathological examinations.

Histopathological examination of the specimen showed follicular hyperplasia in association with islands of mature hyaline cartilage as cartilaginous choristoma in tonsil.

Discussion:

The neck is developmentally complex, with frequent embryologic anomalies. Heterotopic tissue as hamartoma or choristoma is another interesting findings [3]. Cartilaginous choristoma was first described by Berry in 1890 [1, 4]. The age group ranges widely from 10 to 80 years. The other sites at which they occur are pharynx, hypopharynx, oral mucosa and middle ear [5, 6].

Various mechanisms have been suggested for pathogenesis of hetrotopia, that are multi- lineage potential of mesenchymal progenitors cells which were able to differentiate into various mesenchymal lineages, as proposed by Haemel et al. [7]. Chondroid choristomas of the tongue are more common in females, although in palantile tonsil they do not have any sex predilection [6]. Choristoma of the tonsil appears to be a developmental anomaly associated with the second pharyngeal arch and could be one of the cause of recurrent tonsillitis. Few other opine that extraskeletal proliferation of cartilage in oral cavity and maxillofacial soft tissue probably reflects the multipotential nature of primitive mesenchymal cells, which may be stimulated to grow by trauma, irritation, or inflammation [8]. Presence of choristoma in the tonsil is extremely rare. Erkilic et al., in their study of routine tonsillectomy specimens found a 3 % incidence of cartilage in the tonsillar tissue.

In view of recurrence seen in certain extraoral cases, excision should involve removal of perichondrium, because it may have the potential to develop new cartilage [9, 10].

To conclude, choristomas are rare entity and of academic interest. Although they are rare, a high index of suspicion for choristomas is needed, when a patient with recurrent tonsillitis is being evaluated.

[1]Department of Pathology, Kathmandu University School of Medical Sciences. [2]Department of Otolaryngology, Kathmandu University school of Medical Sciences, Dhulikhel, Nepal
Correspondence to: Sujan Sharma, Department of Pathology, Kathmandu University School of Medical Sciences

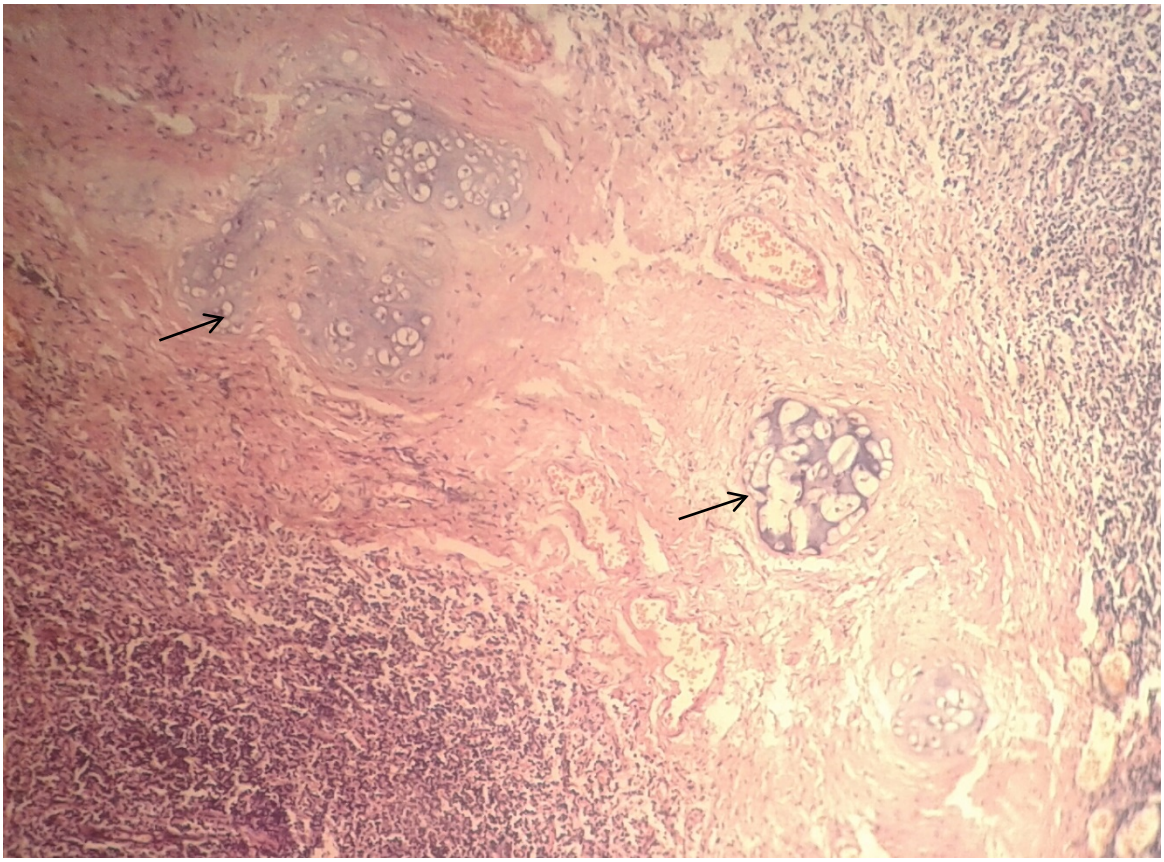

Fig 1. Microscopic picture lymphoid hyperplasia along with islands of mature hyaline cartilage.

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Editor-in-Chief to avoid conflict with scheduled reviews invited by the Editorial Board. They should address new topics or trends in clinical biochemistry or related fields. Consensus recommendations or guidelines on the use of laboratory test for clinical practice will be considered if they are compiled by a recognized organization or expert panel (e.g. IFCC, IUPAC, AACC, etc.) Please contact the Editor-in-Chief for consideration. The responsibility for such material remains with the originating body

Essential title page information

Title. Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.

Author names and affiliations. Where the family name may be ambiguous (e.g., a double name), please indicate this clearly. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.

Corresponding author. Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. **Ensure that phone numbers (with country and area code) are provided in addition to the e-mail address and the complete postal address. Contact details must be kept up to date by the corresponding author.**

Present/permanent address.If an author has moved since the work described in the article was done, or was visiting at the time, a 'Present address' (or 'Permanent address') may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

Abstract

A concise and factual abstract is required. The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if essential, then cite the author(s) and year(s). Also, non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself. Abstract should be presented in a structured format and should not exceed 250 words. The following headings should be included followed by a colon:

a) Background
b) Methods
c) Results
d) Conclusion

Keywords

Immediately after the abstract, provide a maximum of 10 keywords for full papers, or 5 keywords for Short Communications, using American spelling and avoiding general and plural terms and multiple concepts (avoid, for example, "and", "of"). Please use terms from the most current issue of medical subject headings of *Index Medicus*. The key words should cover precisely the contents of the submitted paper and should give readers sufficient information as to the relevance of the paper to his/her particular field. Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.

Maximum length of submissions

Original Article: Full length articles should not exceed 3000 words, maximum 40 references, and up to 6 tables and/or figures.

Short communications: 1500 words of text, maximum 15 references, and two illustrative items (Tables and/or Figures).

Abbreviations

Define abbreviations that are not standard in this field in a footnote to be placed on the first page of the article. Such abbreviations that are unavoidable in the abstract must be defined at their firstmention there, as well as in the footnote. Ensure consistency of abbreviations throughout the article.

Acknowledgements

Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here

those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

Footnotes

Footnotes should be used sparingly. Number them consecutively throughout the article. Many Word processors build footnotes into the text, and this feature may be used. Should this not be the case, indicate the position of footnotes in the text and present the footnotes themselves separately at the end of the article. Do not include footnotes in the Reference list.

Table footnotes

Indicate each footnote in a table with a superscript lowercase letter.

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Electronic artwork
General points
• Make sure you use uniform lettering and sizing of your original artwork.
• Preferred fonts: Arial (or Helvetica), Times New Roman (or Times), Symbol, Courier.
• Number the illustrations according to their sequence in the text.
• Use a logical naming convention for your artwork files.
• Indicate per figure if it is a single, 1.5 or 2-column fitting image.
• For Word submissions only, you may still provide figures and their captions, and tables within a single file at the revision stage.
• Please note that individual figure files larger than 10 MB must be provided in separate source files.

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Regardless of the application used, when your electronic artwork is finalized, please 'save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below):

EPS (or PDF): Vector drawings. Embed the font or save the text as 'graphics'.
TIFF (or JPG): Color or grayscale photographs (halftones): always use a minimum of 300 dpi.
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Number tables consecutively in accordance with their appearance in the text. Place footnotes to tables below the table body and indicate them with superscript lowercase letters. Avoid vertical rules. Be sparing in the use of tables and ensure that the data presented in tables do not duplicate results described elsewhere in the article.

The Editor-in-Chief, on accepting a manuscript, may recommend that additional tables containing important backup data, too extensive to be published in the article, may be published as supplementary material. In that event, an appropriate statement will be added to the text.

Submit such tables for consideration with the manuscript.

References

Citation in text

Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Any references cited in the abstract must be given in full. Unpublished results and personal communications are not recommended in the reference list. Citation of a reference as 'in press' implies that the item has been accepted for publication and a copy of the title page of the relevant article must be submitted.

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As a minimum, the full URL should be given and the date when the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately (e.g., after the reference list) under a different heading if desired, or can be included in the reference list.

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Please ensure that the words 'this issue' are added to any references in the list (and any citations in the text) to other articles in the same Special Issue.

Reference formatting

There are no strict requirements on reference formatting at submission. References can be in any style or format as long as the style is consistent. Where applicable, author(s) name(s), journal title/book title, chapter title/article title, year of publication, volume number/book chapter and the

pagination must be present.. If you do wish to format the references yourself they should be arranged according to the following examples:

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Text: Indicate references by number(s) in square brackets in line with the text. The actual authors can be referred to, but the reference number(s) must always be given.

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Examples:

Reference to a journal publication:

1. Regmi P, Malla B, Gyawali P, Sigdel M, Shrestha R, Shah DS, Khanal MP. Product of serum calcium and phosphorus (Ca x PO4) as predictor of cardiovascular risk in predialysis. Clinical Biochemistry 2014;47:77-81.

Reference to a book:

2. Strunk Jr W, White EB. The elements of style. 4th ed. New York: Longman; 2000.

Reference to a chapter in an edited book:

3. Mettam GR, Adams LB.How to prepare an electronic version of your article. In: Jones BS, Smith RZ, editors. Introduction to the electronic age, New York: E-Publishing Inc; 2009, p. 281–304.

4. Scientific and Technical Report: - WHO. Control of the leishmaniasis 1990, Technical Report Series 793.

5. Papers accepted for publication: Hirai K, Takagi E, Okuno Y et al. Status of polyunsaturated fatty acids in serum of persons aged 10-72 in Nepal. Nutr Res (in press).

Submission checklist

The following list will be useful during the final checking of an article prior to sending it to the journal for review. Please consult this Guide for Authors for further details of any item.

Ensure that the following items are present:

One author has been designated as the corresponding author with contact details:
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• Telephone

All necessary files have been uploaded, and contain:
• Keywords
• All figure captions
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Further considerations
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