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Fairness of Oral Assessment

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Assessment plays a crucial role in any curriculum. Oral assessment has long history in the certification process of medical education in most countries. Medical educators around the world have successfully used many different methods of assessing learners, both written and oral.[1] Oral assessment is a form of assessment, where a set of stimulus questions are developed that address critical areas of knowledge or sets of abilities related to a competency or set of competencies. In oral assessment, students are expected to respond verbally in their own words, which allow an assessment of student's depth of comprehension, and capacity to apply knowledge and insights to different situations.[2] Responses to the questions are assessed using a rating scale or scoring system. Oral assessment contains a high degree of flexibility; the teachers have possibility to choose and change the rules and procedure of assessment.[2] The complexity of oral assessment as an examination format raises concerns about its validity, reliability and fairness of its procedure for the award of certification after completion of a course. Validity "is the degree to which a test 'truly' measures what it is intended to measure" and it is the first priority of any assessment.[3] On the other hand, reliability relates to consistency in measurement, that is, scores derived from a reliable assessment tool are similar across assessment events.[4] The oral assessment format facilitates instructors to test the student on all five domains of Bloom's taxonomy.[5] To minimize the inconsistency and inequality of oral assessment among examiners, most authors suggested to develop a standardized format which have better validity and reliability, with less susceptibility to gender or cultural bias and to ensure its fairness as structured oral exam (SOE).[6] They also suggested that examiners need to familiar with the new system in a short time training programme that favour to ensure homogenous scores among different raters. It is highly recommended to implement standards, benchmark, and performance indicators for effective oral assessment.[7]

Lastly, the development of assessment instruments that are valid, reliable and fair is not an easy task. This involves a process of lengthy discussions, modification, support and encouragement among the people engaged in the educational system, mainly, the administration, teachers, and students.

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ORIGINAL ARTICLE

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Anthropometric and other Determinants of Peak Bone Mineral Density: A Hospital Based Study among Healthy Bangladeshi Volunteers

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ABSTRACT

Introduction: *The peak bone mineral density (PBM) status is one of the most important determinants of future development of osteoporosis. As such data on bone mineral density (BMD) and related information is important in the assessment and prevention of osteoporosis.* ***Objectives:*** *To study the anthropometric and other determinants of PBM status, in a hospital based healthy Bangladeshi volunteers.* ***Methods****: This cross-sectional observational study was conducted from July to September 2014. A total of 207 young (21-39 years) healthy volunteers of both genders were recruited. The anthropometric parameters, dietary evaluation to quantify calcium and protein intake, bone mineral density (BMD) by dual energy X-ray absorptiometry (DXA) was measured. PBM at different sites for both male and female were determined from quadratic regression model of BMD on age.* ***Results:*** *PBM (gm/cm^2) values for female of lumbar spine (L1-L4) and total femur were found 1.147 and 1.019 respectively and the age of attainment of those peaks were 30, and 29 years respectively. For male those values were 1.148 and 1.091 (gm/cm^2) and 28 and 21 years, respectively. Mean intake of calcium (303±202 mg/day) and protein (54.17±16.69 gm/day) was lower than the recommended daily allowances (RDA). On multiple regressions analysis, weight was the most significant predictor of BMD of total femur in female subjects. In male, age was the most significant negative predictor of BMD of both lumbar spine and total femur. On the basis of BMD, Z-score of lumbar spine, 4.9% female and 11% male subjects suffered from low bone mass.* ***Conclusion:*** *A significant proportion of clinically healthy young subjects suffered from low bone mass. Intervention at various levels may help to improve PBM and prevent osteoporosis thereby.*

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INTRODUCTION

Peak bone mineral density (PBM) is the bone mass during stable period following growth and accrual of bone mass, prior to subsequent bone loss.[1] It is the average maximum bone mass achieved by healthy sex and race-matched adults which is normally achieved after puberty. Bone mass at any time in life is a function of the PBM and the amount lost from maturity.[2] Genetic, environmental or life style factors contribute to PBM accrual.[3,4] Genetic factor is the most important among them; about three-fourth of the variance of PBM in a population is determined by genetic factor. This heritable component of PBM accrual is assumed to be polygenic in nature.[5] Environmental and life style factors (like, calcium and protein intake, vitamin D status, exposition to sunlight, physical activity, nutrition, effect of drugs) account for the rest.[6] Up to 90% of PBM is acquired by age 18 in girls and by age 20 in boys, which makes youth the best time to "invest" in one's bone health.[7] High levels of physical activity and good calcium intake during childhood and puberty can help achieve maximal peak bone mass.[8]

Osteoporosis, the most common metabolic bone disease affects one in three women and one in five men over the age of 50 years.[9] Osteoporosis is directly related to improper accrual of PBM; studies estimated that 60% of the risk of osteoporosis can be explained by low PBM. Subsequent bone loss accounts for the remaining risk (of osteoporosis).[10] Thus, even though most osteoporotic fractures occur in elderly people, the risk of osteoporosis may be profoundly affected by events in early life. Osteoporosis related fracture is associated with increased mortality, concomitant morbidity, and reduced quality of life.[11]

Determination of PBM and the time of its attainment are essential for targeting interventions aimed at achieving optimal PBM and thus reducing the risk of future osteoporosis. In Bangladesh, till date we don't have population based information on PBM. This study was aimed to determine BMD at different sites, PBM at each site along with some of its important determinants among healthy hospital based young individuals.

METHODS

This cross-sectional observational study was conducted in the Department of Rheumatology, Bangabandhu Sheikh Mujib Medical University (BSMMU) in 2014. Young apparently healthy 207 hospital based subjects (doctors, hospital stuffs e.g. stuff nurse, ward boy, porter, cleaner, laboratory personnel , security guards and attendants of patients), aged 21-39 years of both genders (female 107, male 100), who had no apparent constraints to bone growth and mineralization were recruited. After obtaining the informed written consent of the study subjects, study questionnaire was served. Pregnant and lactating women, current tobacco user, persons with medical disorders or those who had received drugs likely to affect bone mineral density (BMD) in last 2 years, and those who had sustained a fracture within the same period were excluded from the study. Subjects who were on calcium and vitamin D supplements for more than 3 months were also excluded from the study.

After screening, BMD of 200 subjects was done (female 102, male 98, dropped out 7) by dual energy X-ray absorptiometry (DXA, by Lunar Prodigy PA+350263 scanners by 'GE Healthcare') of lumbar spine (L1-4), neck of femur (NOF) and total femur, was done. Quality control of the scanner was done by phantom scan, on a daily basis, during the study period. Verification of phantom mean BMD was done on the densitometer followed by corrective action threshold where needed, according to "The International Society for Clinical Densitometry" (ISCD) guideline.[12] Anthropometric parameters including weight, standing height, body mass index (BMI) was obtained.

Food recall interview was done by an experienced nutritionist. Subjects stated about the food and beverages they consumed in the last 24 hours (24 hour food recall), from which daily cal-

cium intake (mg/day), and elemental protein intake (gm/day), were determined. The calcium and protein content of more than 80 food items, which includes staple food, pulses, vegetables, fruits, animal protein, and junk food, were derived from reference book.[13] Total daily calcium (mg/day), and protein (gm/day) intake was calculated from coded food chart, after serving study subjects real food items, photographs or dough, followed by measuring /assessing the amount consumed. Any inconsistency was dealt with by re-interviewing.

BMD was expressed as gm/cm^2 and Z- score, which is a comparison of the patient's BMD to an age-matched 'normal reference population'. Value of Z- score lower than two standard deviations from the mean was considered as osteopenia.[14] Osteopenia with ≥1 fragility (low impact) fracture was considered as osteoporosis. The reference population is a 'Combined National health and nutrition examination survey (NHANES III)'/Lunar based 'Asia' population whose data base was incorporated in the DXA densitometer.[15] The Z-score thus determined were entered. All scans were carried out on the same machine by the same operator and analyzed by the same software. Simple descriptive measures like percentage, mean and standard deviation of different variables were used. Peak BMD at different sites for both male and female was determined from quadratic regression model of BMD on age. Student's t test (unpaired) was used to examine differences between the mean BMD levels of 2 different age groups. Stepwise multiple regression analysis was performed using BMD as the dependent variable and others as the independent variables, to determine predictors of BMD. All data analysis was done using the SPSS/PC statistical software package.

RESULTS

A total of 230 individuals participated in the study, 23 were excluded. Baseline demographic data of remaining 207 was collected (Table I). Bone mineral density was done by DXA of 200 subjects. Mean age of the study subject was 28. 99±5.39 years. The body mass index (BMI) of female (24.28±3.78 kg/m^2) was higher than that of male (23.72±3.42 kg/m^2). Mean monthly income was 12354±9329 and 12874±9359 taka for female and male respectively. Dietary intake of calcium (mg/day) by male and female were 312±216, and 294 ±189 respectively. Protein intake (gm/day) by them was 60.72±18.11 and 48.05±12.53 respectively. The difference between male and female protein intake was statistically significant.

Table I: Baseline characteristics of the study subjects (n-207)

Characteristics of the study subjects	Female (n-107) Mean±SD	Male (n-100) Mean±SD	Total subjects (n-207) Mean±SD
Age (years)	28.92±5.34	29.07±5.48	28. 99±5.39
Height (cm)	152.31±5.96	164.52±6.58	158.35±8.75
Weight (kg)	56.5±10.65	64.15± 8.5	60.28±10.36
BMI (kg/m^2)	24.28±3.78	23.7±3.42	24.01±3.61
Monthly income (Tk.)	12354±9329	12874±9359	12627±9323
Calcium intake (mg/day)	294±189	312±216	303±202
Protein intake (gm/day)	48.05±12.53	60.72±18.11	54.17±16.69

BMD, its trend at different sites, and peak

Bone mineral density (gm/cm^2) of L1-L4 was 1.128±0.118 for male and 1.133±0.114 for female were comparable, but BMD at other sites are better for male (1.006 vs. 957 neck of femur, 1.049 vs. 1.002 total femur). Z-score at all measured sites for female was better than those for male (Table II). Overall, BMD was observed better at L1-L4 (Figure 1), but the Z-score was observed better at total femur (-0.1±0.9).

Table II: Bone mineral density by dual energy X-ray absorptiometry and corresponding Z- score of the study subjects (n-200)

Investigations BMD (DXA), n=200	Female (n-102) Mean ± SD	Male (n-98) Mean ± SD	Total subject (n-200) Mean ± SD	*p
BMD, L1-L4 (gm/cm^2),	1.133 ± 0.114	1.128 ± 0.118	1.131± 0.115	0.803
L1-L4 Z-Score	-0.40 ± 0.9	-0.74 ± 0.9	-0.5 ± 0.9	0.011
BMD NOF(gm/cm^2)	0.957± 0.136	1.006 ± 0.126	0.9 81± 0.133	0.008
NOF Z-Score	-0.46 ± 0.9	-0.48 ± 0.9	-0.4 ± 0.9	0.870
BMD, Total femur(gm/cm^2)	1.002 ± 0.125	1.049 ± 0.122	1.025± 0.125	0.007
Total femur Z-Score	0.01 ± 0.9	-0.29 ± 0.8	-0.1 ± 0.9	0.018

* Student's 't' test (unpaired)

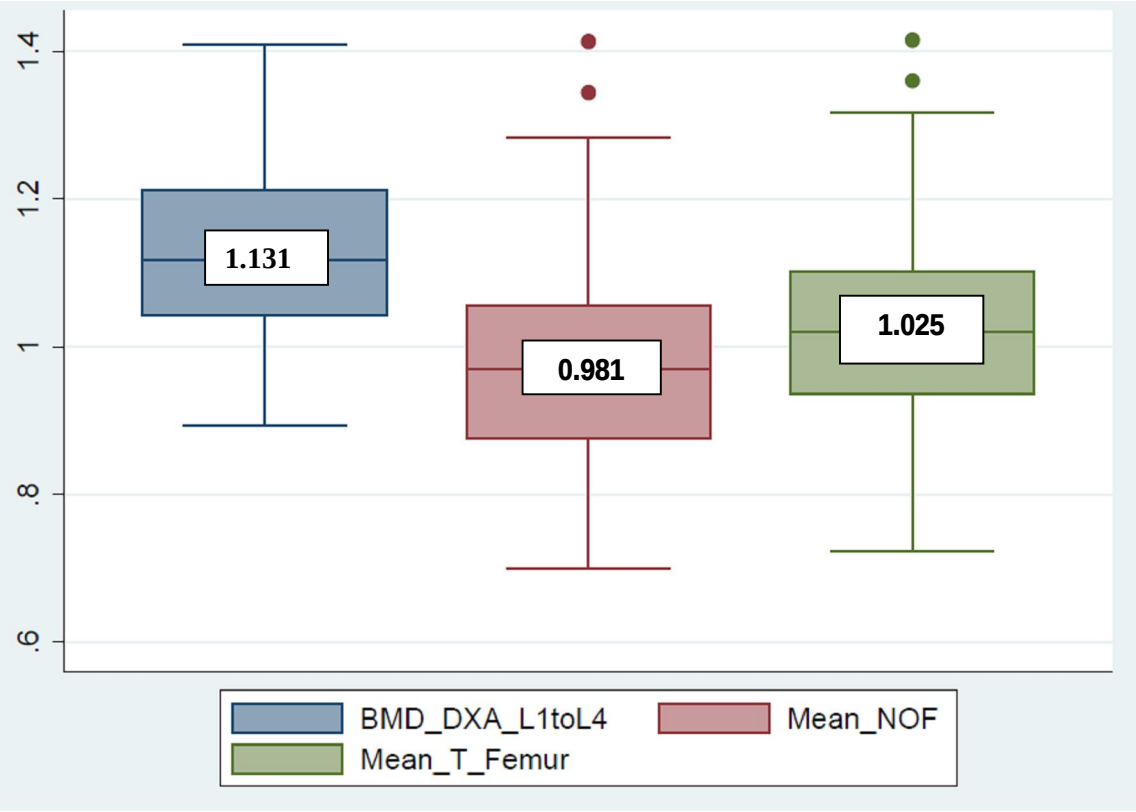

Figure 1: Box plot of mean BMD (DXA) values (of all study subjects)

Computer based quadratic regression model for BMD values on age in both female and male study population was plotted. Based on regression analysis of different BMD values on age, peak bone mineral density (PBM) value and age of its attainment were determined. For female PBM value at L1-L4, NOF, total femur were 1.147±0.016, 0.982±0.019, 1.019±0.017 (gm/cm^2), respectively and the age of attainment of those peaks were 30, 29, and 29 years. For male those figures were, 1.148±0.016, 1.097±0.028, 1.091± 0.028 (gm/cm^2) and 28, 21 and 21 years respectively. Predicted PBM values and the age of attainment of peaks were shown in the Table III. Quadratic regression model of BMD on age at L1-L4 for female and male were depicted in Figure 2 and Figure 3 respectively.

Table III: Predicted peak bone mineral density value and the age of attainment of peak of the study subjects (n- 200)

Predicted peak BMD (gm/cm^2)	Gender Female-102 Male -98	Predicted value ± SE	95% CI	Age of attainment of peak BMD
BMD L1 to L4 predicted peak	Female	1.147±0.016	(1.115 , 1.180)	30
	Male	1.148±0.016	(1.115 , 1.181	28
BMD mean NOF predicted peak	Female	0.982±0.019	(0.943 , 1.020)	29
	Male	1.097±0.028	(1.042 , 1.152)	21
BMD total femur predicted peak	Female	1.019±0.017	(0.984 , 1.054)	29
	Male	1.091±0.028	(1.036 , 1.147)	21

SE= Standard error, CI =confidence interval.

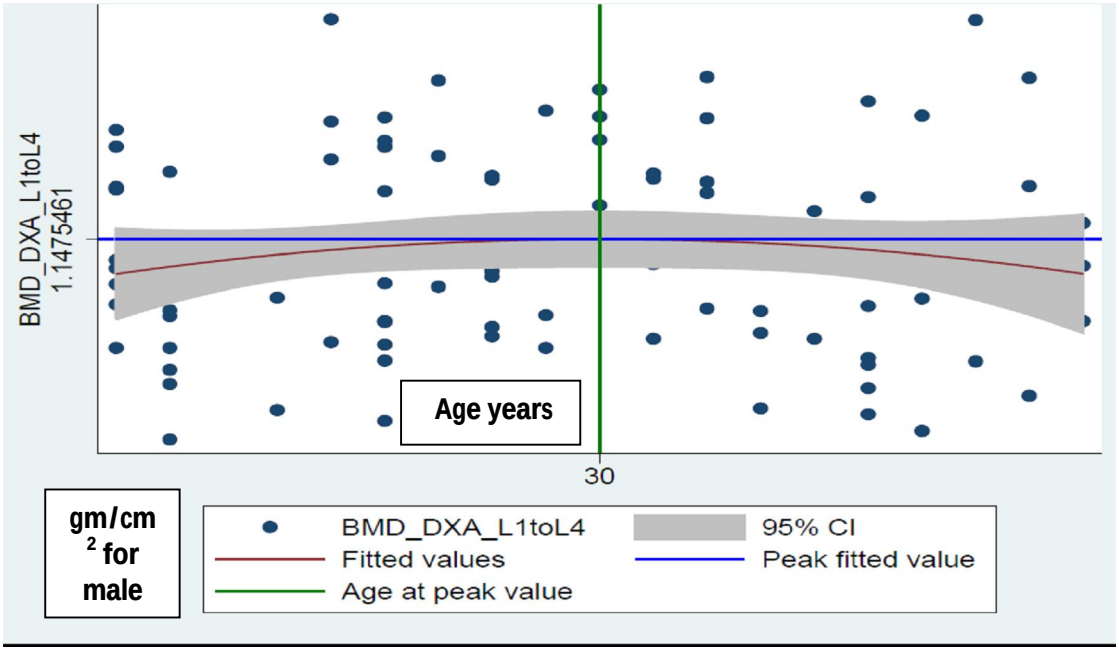

Figure 2: Quadratic regression model of BMD on age for female L1-L4, peak BMD was 1.147±0.016, age of attainment 30

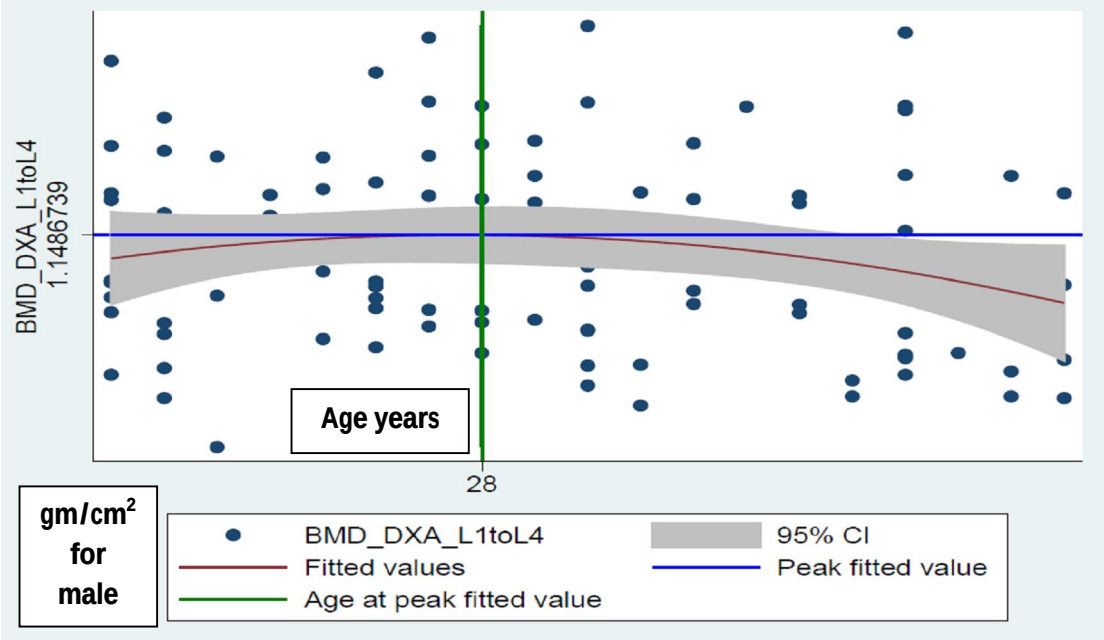

Figure 3: Quadratic regression model of BMD on age for male L1-L4, peak BMD was 1.148±0.016, age of attainment 28

Predictors of BMD in regression analysis:

On multiple regressions analysis of BMD on predictor variables (e.g. weight, height, protein intake), weight was the most significant predictor of BMD of neck of femur and total femur for female subjects (*p*-0.00 and *p*-0.00 respectively). For male, age was the most significant and most consistent negative predictor of BMD at all the measured sites (*p*-0.009, *p*-0.028, *p*-0.041, for L1-L4, NOF, and total femur respectively) (Table IV and V).

Table IV: Multiple regression analysis of bone mineral densityscore on predictor variables (female subjects, n-102)

Independent Variable	Dependent Variable: BMD L1-L4 (gm/cm2)			Dependent Variable: BMD Neck of femur (gm/cm²)			Dependent Variable: BMD Total femur (gm/cm²)		
	Reg. coeff. (b)	*t*	*p*-value	Reg. coeff. (b)	*t*	*p*-value	Reg. coeff. (b)	*t*	*p*-value
Age	0.00	0.09	0.927	-0.004	-1.56	0.124	-0.005	-1.90	0.062
Weight	0.002	1.43	0.157	0.006	3.83	0.000	0.007	4.57	0.000
Height	0.005	1.82	0.074	0.001	0.23	0.816	-0.002	-0.88	0.380
Protein	0.001	0.72	0.477	0.002	1.83	0.073	0.002	1.47	0.148
(Constant)	0.185	0.48	0.632	0.501	1.17	0.248	1.012	2.44	0.017
R Square	0.192			0.286			0.288		

Reg. coeff.=regression coefficient

Table V: Multiple regression analysis of bone mineral density score on predictor variables (male subjects, n-98)

Independent Variable	Dependent Variable								
	BMD (DXA) L1-L4 (gm/cm2)			Mean-NOF			Mean-T. Femur		
	Reg. coeff. (b)	*t*	*p*-value	Reg. coeff (b)	*t*	*p*-value	Reg. coeff. (b)	*t*	*p*-value
Age	-0.019	-2.90	0.009	-0.014	-2.37	0.028	-0.016	-2.19	0.041
Weight	0.007	1.79	0.09	-0.001	-0.32	0.749	0.001	0.15	0.881
Height	0.002	0.52	0.611	0.006	1.34	0.195	0.007	1.43	0.169
Protein	0.002	1.33	0.198	0.001	0.48	0.638	0.002	0.99	0.336
(Constant)	1.016	1.48	0.157	0.552	0.88	0.389	0.319	0.43	0.675
R Square	0.403			0.455			0.350		

Z-score and osteopenia/osteoporosis

Z-score of ≤ -2 is considered as "low bone mass" or bone mass "below the expected range for age". According to that at L1-L4, NOF and total femur, Z- score ≤ -2 was observed in 4.9%, 2.9% and 0.98% in female and 11.2%, 3% and 2% in male subjects respectively. When total subjects are taken into account, the values (below the expected range) were 8%, 3% and 1.5 %, respectively of those sites. Z-score based distribution of study subjects, were shown in Table VI.

Table VI: Z- score based distribution of study subjects (n-200)

BMD sites	Low bone mass "below the expected range for age"(Z-score ≤-2)			
	Female n-102 (%)*	Male n-98 (%)*	Total n-200 (%)*	**p* value
Lumbar spine (L1-L4)	05 (4.9)	11 (11.2)	16 (8)	0.17
Neck of femur	03 (2.9)	03 (3)	06 (3)	0.70
Total Femur	01 (0.98)	02 (2)	03 (1.5)	0.99

* Z proportion test

Comparison of BMD with other study and manufacturer's data

Female study subjects in our series were segregated into 2 age groups, then BMD values at L1-L4 and NOF were recalculated after cross calibration of values (from GE Lunar to 'Hologic'),[15] for the purpose of comparison of those values to an Indian study.[16] Recalculated values of our female population for L1-L4 were 14.3% and 9.8% better than those of corresponding Indian study (for 21-30 and 31-40 age groups respectively). The difference was statistically significant. For NOF those values were 5.9% and 0.3% better for those age groups (Table VII).

Table VII: Comparison of bone mineral density with Indian population, in spine and dual neck of femur (Bangladesh n-64, n-38, Indian n-50, n-50)

Age group (year)	Sites	Bangladesh (Female n-64 (21-30) n-38(31-40) **		Indian (Female)[28] n-50(21-30),n-50(31-40)		% difference	*p-value
		Mean±SD	CV	Mean±SD	CV		
21-30	Spine	1.054±0.104	9.9	0.903±0.159	17.6	14.3	0.000
31-40	Spine	1.044±0.119	11.4	0.942±0.1 14	12.1	9.8	0.000
21-30	NOF	0.795±0.121	15.2	0.748±0.214	28.6	5.9	0.141
31-40	NOF	0.775±0.111	14.3	0.773±0.120	15.5	0.3	0.936

*Students t test (unpaired), **(Recalculation from the NHANES Database[15])

Similar comparison to NHANES III[15] population database (both male and female) of total femur showed that there was no significant difference between the values of female subpopulation. But NHANES values for male were 3.9% higher than those of our series. The difference (*p*-0.04) was significant (Table VIII).

Table VIII: Comparison of bone mineral density with NHANES III population at total femur

Gender	Bangladesh (21-30) n-64 (F), n-62 (M)		NHANES (20-29)[15] n-409 (F), n-382 (M)		% diff.	*p-value
	Mean ± SD	CV	Mean±SD	CV		
Female	0.944±0.125	13.2	0.942±0.122	13.0	0.2	1.00
Male	1.002±0.111	11.1	1.041±0.144	13.8	-3.9	0.04

*Students t test, SD= standard deviation, CV = coefficience of variance

DISCUSSION

The peak bone mineral density (PBM) status is one of the most important determinants of the future development of osteoporosis.[17] With the view to measure the PBM, we determined the bone mineral density (BMD) status of 200 study subjects. Different other studies had similar study population[2,18]; some other study had larger population.[19-21] Age range of this study subjects was 21-39 years. Other study had similar (21-40 years)[19] or dissimilar (25-35years)[18,20] study population. Mean age of our study subjects (28.99 years), was comparable to other study.[18,20] Both height and weight of female in this series were lower than those in male and the difference was significant (for both variables *p* - 0.00), although BMI of female, was higher. This observation (higher values for male) was comparable to the Indian series.[20]

Mean daily calcium and protein intake was low in this series (262.66 mg, 52.48 gm), which was below the RDA (400 mg/d and 1gm/kg/day respectively). It was also low in comparison to other studies.[18,20] Significant difference of protein intake between male and female was observed in our series (60.72 gm vs. 48.05 gm, *p*-0.00), which is similar to the Indian study[20] (67.7 gm vs. 50.92 gm). Such a low daily intake may be related to lack of knowledge, and/or poor buying capability or other social factor(s).

Mean BMD (gm/cm^2) by DXA, of all study subjects of L1-L4 was better than other measured sites. Other studies[20,21] also revealed similar higher

BMD trend at that same area. BMD (in gm/cm^2) for male was comparable to or better than female, but Z- score at all measured sites of female was better than male. Discrepancies between gm/ cm^2 and Z-score value of BMD in our series is an interesting observation which might be an indication that 'NHANES III' reference population database is not working well in Bangladesh. We had to apply Z-score, as per ISCD guidelines.[12] (Z-scores, not T-scores, are preferred for BMD reporting in females prior to menopause and in males younger than age 50.)

Ideally, PBM is estimated from a longitudinal study in which a large number of male and female is followed from the age 5 till the age 30, but such a study is not practically feasible; [22] although there are a number of longitudinal study.[1,7,23-26] Our study is a cross sectional one; we found a good number of similar cross sectional study.[2,4,18-21] PBM determination strategy from cross sectional studies also differed from one study to another. Some study protocol categorized male and female population into 2 (or more) different age sub-groups and better mean BMD values for a subgroup was considered PBM and that age (group), considered as the age of attainment of PBM. Shivane's[20] study in this manner, calculated that PBM at some scanned area appeared earlier and at other areas later. Similar method was applied in other studies as well.[18,19,21]

We used a computer based regression model to determine PBM. There are other studies,[22,27] who used similar model. Based on regression of different BMD values on age, peak BMD value and age of its attainment were calculated. PBM at all measured sites in our series were higher for male & the age of attainment were earlier for them (male). For both genders, lumbar peak was higher. Bagher et al.,[21] had similar higher lumber peak (all study population) and earlier age of peak attainment by male. Study conducted in Vietnam[22] had all those PBM values lower than those of our series and age of attainment were earlier for female.

On regression analysis weight for female and negative correlation of age for male, was the most significant predictor of BMD (female 2 sites, male all sites) in our series. Although height didn't predict BMD in our series, Shivane et al.,[20] found height along with weight as the most significant predictors of BMD at all sites. Height, weight, and total body fat were the most significant predictors of BMD by Fuleihan et al.[18] Weight was the most consistent contributor to variance in BMD by Marwaha et al.[19] Age, lean body mass, physical activity and dietary calcium intake accounted for PBM in the study by Suzanne et al.[28]

Z– Score based BMD study revealed that up to 11.2% healthy male and 4.9% healthy female subjects had "low bone mass." (At L1-L4). Comparison of BMD value with that of Ranu P et al.,[16] revealed that, our female subpopulation had up to 14.3% better BMD value than their Indian counterpart. When compared to NHANES III population database for total femur, there was no significant difference between the values of female subpopulation. But our male subpopulation were trailing significantly (p-0.04) by that NHANES database. So, our female subpopulation had comparatively better BMD, although they were trailing by their male counterpart in the study.

Limitations

The study was conducted on a group of population mainly of urban background, which may not be the representative population. As the study was not a longitudinal one, any result of PBM may be an underestimate of the true PBM of the study population. Sampling was not random (rather convenient), which might be subjected to bias. Many other possible determinants of PBM were not studied, like serum calcium, Vitamin D, bone markers, Inorganic phosphate and others. 24 hours food recall may not represent the food habit and average energy consumption of the

study subjects. Determination of PBM requires a healthy population with no constraints to growth, nutrition, and bone mineralization. Low intake of dietary calcium and protein by our population might not represent them as "healthy young population." A bigger sample would have a better representation of the PBM and its determinants.

CONCLUSION

Nutrition status needs to be improved in order to optimize peak bone mineral density and to prevent or retard future development of osteoporosis. Diet (with supplementation) providing adequate protein, vitamin D, calcium, and other elements in the years prior to peak bone mass is very important. To the best of knowledge it was the first population based BMD study in Bangladesh. Results obtained and the observations made in this study may help to generate population based database, and encourage comprehensive study on this field in future.

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ORIGINAL ARTICLE
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Synthesis of Zinc Oxide Nanoparticles using Peel Extract of Citrus paradisi and its Antibacterial Effects

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ABSTRACT

Introduction: *The interaction of nanoparticles (NPs) with biomolecules and microorganisms is an expanding field of research. Zinc oxide nanoparticles (ZnO-NPs) are known to be one of the multifunctional inorganic nanoparticles with effective antibacterial activity. This study aims to determine the antimicrobial efficacy of biologically synthesized ZnO-NPs.* ***Methods:*** *This quasi experimental study was designed to synthesize of ZnO-NPs from zinc sulphate monohydrate ($ZnSO_4.H_2O$) solution using Citrus paradisi peel extract as reducing agent as well as capping agent which being considered a rapid process that requires no toxic chemicals. The characterization of nanoparticles was done by using (Ultraviolet visible) UV-Vis spectroscopy. This biological synthesis guided ZnO-NPs were then studied on Gram negative bacteria like Escherichia coli (E. coli) using disc diffusion method to evaluate its antibacterial activity.* ***Results:*** *The ZnO-NPs containing solution showed distinctive colour change and a sharp peaked Surface Plasmon Resonance (SPR) appeared at 370 nm which suggested formation of nanoparticles. The antibacterial activity of different concentrations of ZnO-NPs, $ZnSO_4.H_2O$ solution and reference drug ciprofloxacin revealed that ZnO-NPs possessed significant antibacterial effect ($p < 0.001$) compared to $ZnSO_4.H_2O$ solution but relatively less antibacterial effect than that of ciprofloxacin.* ***Conclusion:*** *The results depicted that the biologically synthesized nanoparticles have significant antibacterial property. Wide range of antibacterial effects, safety and detailed mechanisms of ZnO-NPs should be further studied thoroughly.*

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INTRODUCTION

Nanotechnology may be defined as the synthesis, characterization, exploration and application of nano-sized materials for the development of science.[1] It has wide range of applications in nanomedicine, nanoelectronics, energy production and consumer products etc. Nanoparticles (NPs) are the particles having at least one dimension less than 100 nm.[2] Smaller the size of particles greater will be surface area compared to their volume which enhances the reactivity of the nanoparticles.[3] The nanoparticles are synthesized through physical, chemical and biological methods. Conventional physical and chemical methods produce some adverse effects like production of toxic byproduct, requirement of high temperature and pressure, more time consumption and expensive etc.[4] Biological method is one of the most preferred method because it is simple, inexpensive, good stability of nanoparticles, less time consuming, non-toxic byproducts and large scale synthesis etc. Moreover, use of plants or part of plants for the synthesis of nanoparticles is ecofriendly.[3,5] Zinc is an important metal of our body. It acts as a cofactor of enzymes. It supports gonadal activity, growth, wound healing and boost immune system. Zinc oxide nanoparticles (ZnO-NPs) are interesting among all metal oxide nanoparticles due to its antibacterial, antifungal, wound healing and UV filtering properties.[6,7] It has also antioxidant, catalytic and anti-diabetic activity. It has longer durability, higher selectivity and heat resistance capacity.[8]

Bacterial infectious diseases are serious health problems that have drawn the public attention worldwide as a human health threat, which extend to economic and social complications. Increased outbreaks and infections by pathogenic strains, bacterial antibiotic resistance, emergence of new bacterial mutations, lack of suitable vaccine and hospital associated infections are global health hazards to humans. So, innovation of new antibiotics is the immense challenge for modern medical science**.** ZnO-NPs produce antibacterial activity by several mechanisms such as by direct interaction with bacterial cell wall, by liberating antimicrobial ions mainly zinc ions (zn^{2+}) and by forming reactive oxygen species (ROS) etc. Interestingly, ZnO-NPs are reported by several studies as non-toxic to human cells.[9] So, this aspect necessitated their uses as antibacterial agents.

Researchers are using biological method for the synthesis of various nanoparticles due to eco-friendly nature. Many researchers used different kinds of plants for the synthesis of ZnO-NPs such as *Citrus paradisi, Citrus aurantifolia,* Seaweeds, aloe vera, *punica granatum,* neem, tamarind, *Calotropis gigantea,* green tea, lemongrass etc.[10] Among these plants *Citrus paradisi* is cheaper and available in local market of Bangladesh. The local name of *Citrus paradisi* is orange.

The biosynthesis of ZnO-NPs and studies on their antimicrobial effects are still in the infancy stage and limited number of works has been reported. So far, this type of study is new in our country. Therefore, it is thought worthwhile to study the synthesis of ZnO-NPs by biological method using *Citrus paradisi* peel extract as reducing as well as capping agent. Antibacterial activity of ZnO-NPs was also evaluated on Gram negative bacteria like *Escherichia coli* by disc diffusion technique.

METHODS

This Quasi Experimental Study was conducted in the Department of Pharmacology and Therapeutics, Rajshahi Medical College, Rajshahi, in collaboration with the Department of Microbiology, Rajshahi Medical College, and Rajshahi during the period of July 2015 to June 2016. Protocol of this study was approved by Institutional Review Board (IRB) of Rajshahi Medical College, Rajshahi.

Preparation of peel extract: At first, within a glass beaker (500 ml) *Citrus paradisi* peel extract was prepared by boiling 100 gm peel in 400 ml of deionized water at 80^0C for 30 minutes. The extract was filtered through Whatman filter paper and stored in refrigerator at 4^0C for further experiments.[11]

Synthesis of ZnO-NPs: The mixture of 90 ml aqueous peel extract of *Citrus paradisi* and 300 ml of 3 mM $ZnSO_4.H_2O$ solution was made in a beaker and stirred for 3 hours at 75-80^0C by Magnetic stirrer with hot plate (Model no. MS-300, Qingdao Tlead International Co., Ltd. China) and kept for observation at room temperature up

to change in colour. The change of colour from yellowish green to light yellow proved for the formation of ZnO-NPs.[11]

UV-Vis Spectral Analysis: The presence of ZnO-NPs **in** the prepared solution was analyzed by UV-Vis spectroscopy using spectrophotometer (Model 340, Sequoia-Turner Corporation, Germany). Absorbance of prepared solution was measured repeatedly after 01 hour, 24 hours, 07 days and 21 days to see the stability of ZnO-NPs. The scanning range for the samples was 350-440 nm.[12] Baseline correction of the spectrophotometer was carried out by using a blank reference.

Antibacterial Activity of ZnO-NPs: The antibacterial activity of ZnO-NPs, $ZnSO_4.H_2O$ solution and Ciprofloxacin were studied by Disc diffusion method on *Escherichia coli* in Mueller-Hinton Agar media (Himedia, Mumbai, India). Twenty four hours fresh cultures were prepared and the standardized (McFarland No. 0.5) inoculum was made and used for the antibacterial study.[3] By using micropipette 10 µg, 20 µg, 30 µg, 40 µg and 50 µg of ZnO-NPs and $ZnSO_4$. H_2O solution was added in sterile paper discs (5mm) made of Whatman filter paper. For 01 µg of $ZnSO_4$, 02 µL of $ZnSO_4$ solution and for the 01 µg of ZnO-NPs, 01 µL of ZnO nanosolution were put into Whatman paper disc**.** Disc containing ZnO-NPs, $ZnSO_4.H_2O$ solution and Ciprofloxacin were placed on solidified agar plates with the help of a sterile forceps.The cultured agar plates were incubated at 37°C for 24 hours. The diameter of the Zones of inhibition including the diameter of discs were measured after 24 hours of incubation with transparent millimeter ruler.[7]

Statistical analysis:

The results were calculated as mean±standard deviation (SD). Data of zone of inhibition of *E. coli* created by ZnO-NPs and $ZnSO_4.H_2O$ (aq) solution were compared using paired *t*-test. A *p*-value less than 0.05 were considered statistically significant.

RESULTS

Synthesis of ZnO-NPs:

Establishment of ZnO-NPs was proved by the change of colour from yellowish green to light yellow.

UV-Vis Spectral Analysis

Absorption spectra of ZnO-NPs had a sharp peak and absorbance maxima at 370 nm. There was no significant change observed in peak position for 1st and 21st day (Figure 1).

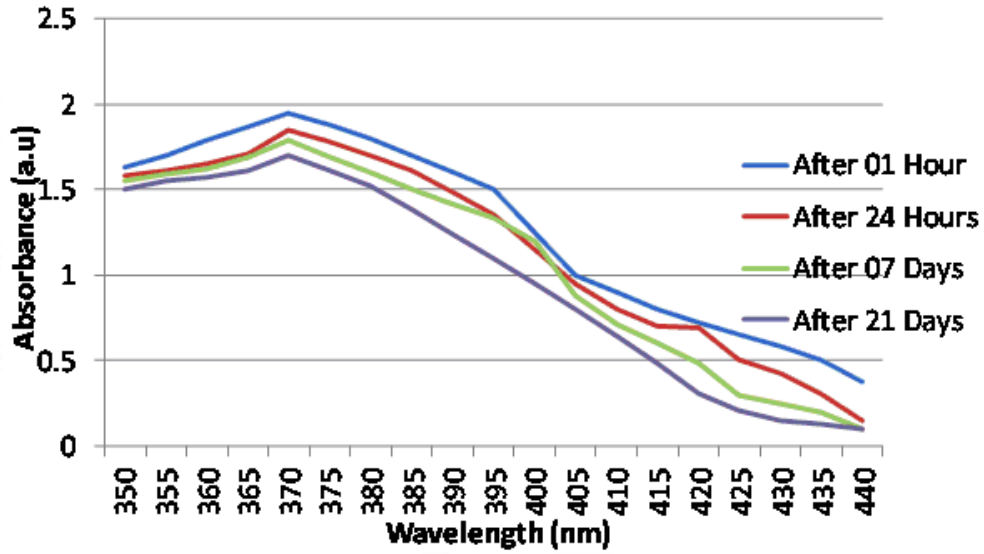

Figure 1. Showing UV-Vis spectra of synthesized ZnO-NPs using *C. paradisi* peel extract

Antibacterial Activity of ZnO-NPs

Zone of inhibition produced by ZnO-NPs, $ZnSO_4.H_2O$ and Ciprofloxacin are shown in Table I. Results were expressed as Mean±SD.

Ciprofloxacin (5 mcg/disc) produces Zone of inhibition 20.83±2.14 mm, which was greater than ZnO-NPs and $ZnSO_4.H_2O$ solution. On the other hand, ZnO-NPs exhibited greater Zones of inhibition than $ZnSO_4.H_2O$ at different strengths. Highest activity of ZnO-NPs was found at the concentration of 50 µg (10.26±0.65 mm). No Zone of inhibition was found in control (Peel extract/ Deionized water) solution. Statistical analysis proved that ZnO-NPs have got significant antibacterial activity ($p< 0.001$) as compared to $ZnSO_4.H_2O$ at all strength.

Table I. Showing Zones of inhibition found in *E. coli* cultures

Concentrations (µg/disc)	Zones of inhibition (mm) (Mean±SD)			
	$ZnSO_4.H2O$	ZnO-NPs	Ciprofloxacin (5 µg/disc)	Control
10 µg	00±00	5.1±0.17		
20 µg	5.15±0.12	6.8±0.78		
30 µg	5.67±0.37	7.53±0.7	20.83±2.14	00
40 µg	6.00±0.63	8.55±0.67		
50 µg	6.62±0.43	10.26±0.65		

$p- < 0.001$

DISCUSSION

Green synthesis of ZnO-NPs from plant extract is better than others.[5] Plants are easily available, safe and nontoxic, in most cases have a broad variety of metabolites. That can aid in reduction of metallic ions quicker than others. Nanoparticles exhibit different colour in aqueous solution due to excitation of surface plasmon vibration.[1,13] In the ZnO-NPs, electrons oscillate collectively. These oscillations affect how light interacts with the nanoparticles. The specific oscillations depend on the particles size and shape. So particles of different sizes have different colours in different surface plasmon absorption peak. Mishra et al.[5] found brownish yellow coloured ZnO-NPs with surface plasmon absorption peak at 364 nm. They used peel extract of *Punica granatum* for the synthesis of ZnO-NPs. Pale-white coloured ZnO-NPs with surface plasmon absorption peak at 325 nm was observed by Senthilkumar et al.[3] They used green tea for the synthesis of ZnO-NPs. Another study done by Ramesh et al.[6] were found light white coloured ZnO-NPs with surface plasmon absorption peak at 208 nm and 215 nm. They used *Citrus aurantifolia* for the synthesis of ZnO-NPs. Rajamanickam et al.[1] stated brown coloured ZnO-NPs with surface plasmon absorption peak at 310 nm. They used Actinomycetes for the synthesis of ZnO-NPs. No peak was observed due to presence of impurities in NPs. Widening of peak indicates very small sized particles.[14] We have found light yellow coloured ZnO-NPs with surface plasmon absorption peak at 370 nm wavelength. Our observation is in agreement with the other study reported by Kumar et al.[11]

UV-vis spectroscopy is one of the most widely used techniques for characterization of nanoparticles. The absorption phenomenon shown by the nanoparticles is due to surface plasmon resonance. The position and shape of plasmon absorption of nanoparticles were strongly dependent on the particle size, dielectric constant and surface

absorbed species. Nanoparticles of various shapes and sizes, from approximately 40 to 120 nm, having colours ranging from violet ~400 nm to red ~700 nm wavelength, were characteristics.[15] Surface plasmons are essentially the light waves that are trapped on the surface because of their interaction with the free electrons on metal. When metal nanoparticles are embedded in dielectric media and specimens re-exposed to electromagnetic radiation, Surface Plasmon Resonance (SPR) absorption band is observed at a specific wavelength depending upon the nature of metal, size of the particles and distribution.

In present study, ZnO-NPs exhibited a single and well defined peak in the absorbance spectrum with maximum absorbance at 370 nm which corresponds to characteristic SPR of ZnO-NPs. Several studies[1,3,5] have observed only single absorption peak like us. On the other hand, Ramesh et al.[6] and Meruvu et al.[14] have founded double and twelve surface plasmon absorption peak respectively.

The synthesized ZnO-NPs were stable without shifting the surface plasmon absorbance band observed at 1st, 7th and 21st day. But after 21 days stability was lost. Stable SPR peak indicates that new particles do not aggregate.[16] Mishra et al.[5] reported synthesis of ZnO-NPs by peel extract of *Punica granatum* was more stable than that of us. Their NPs were stable more than six months.

Therefore, the overall findings concluded that synthesis of ZnO-NPs using peel extract of *Citrus paradisi* were roughly spherical in shape and having size about 70 nm and stable for 21 days. Further study should be done to evaluate the size and shape of NPs.

In this study, the antibacterial activity of ZnO-NPs was tested against common pathogenic organism like Gram negative bacteria *Escherichia coli.* Zone of inhibition is the only criterion which has been used to compare the activity. The anti-bacterial activity of ZnO-NPs showed concentration dependent activity. Though ZnO-NPs exhibited lower zone of inhibition than Ciprofloxacin, ZnO-NPs created significantly greater bacterial zone of inhibition and appeared sensitive compared to $ZnSO_4.H_2O$. The control solution of peel extract and deionized water did not show any antibacterial activity in *E. coli* cultures. The diameters of zone of inhibition in the agar plate were measured in mm and summarized in Table I.

In a similar study done by Senthilkumar et al.[3] evaluated concentration dependent activity of ZnO-NPs on Gram positive and Gram negative bacteria prepared from *Camellia sinensis*. They obtained no zone of inhibition in *E. coli* cultures using 10 µg/disc and 20 µg/disc ZnO nano solution. But we have found 5.1 mm and 6.8 mm zone of inhibition with the concentrations of 10 µg/disc and 20 µg/disc respectively. It was may be either due to structural differences or due to resistant organism. Another study conducted by Prasad et al.[17] evaluated concentration dependent activity of ZnO-NPs on Gram negative bacteria like *E. coli* prepared by combustion method. They showed that 18 mm zone of inhibition in *E. coli* cultures was found at a dose of ZnO-NPs (50 µg/disc), which was possibly due to very small size (30 nm) and highly pure nanoparticles. But we have found 10.26 mm zone of inhibition at same concentration may be due to crude compound and different methods of synthesis. Namasivayam et al.[18] have found greater zone of inhibition (30 mm) with the dose of 50 µg/disc than us and Prasad et al.[17] It might be due to different method of synthesis (chemical method) and smaller particle size than us. The small size of metallic nanoparticles ensures that a significantly large surface area of the particles is in contact with the bacterial effluent. Considering a hypothetical case with spherical particles of uniform size, a reduction in the particle size from 10 m to 10 nm will increase the contact surface area by 10^9.

The exact antibacterial mechanism of ZnO-NPs is not clearly known. The smaller size of NPs facilitates easy entry into the microbial cell mem-

brane, produce broad contact with microorganism and enables inhibition mechanisms inside the cell. ZnO-NPs generate hydrogen peroxides which chemically interact with membrane protein and lipid bilayers. The antimicrobial activities of these NPs may involve both the production of reactive oxygen species (ROS) and the accumulation of NPs in the cytoplasm on the outer membranes. ROS causes membrane dysfunction and cell death by oxidizing the membrane lipids.[3,19] Xia et al.[20] have suggested that smaller sized NPs can enter the mitochondria of cells through various pathways and thereby induce oxidative stress and cell death via apoptosis.

CONCLUSION

In the present study, zinc oxide nanoparticles were successfully obtained by *Citrus paradisi* peel extract assisted synthesis. Significant colour change and UV-Vis spectroscopy suggested the formation of nanoparticles. Those zinc oxide nanoparticles showed significant antibacterial activity in compared to zinc sulphate monohydrate. Characterization, wide range of antimicrobial activity, mechanism of actions and safety profile of zinc oxide nanoparticles may be recommended.

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ORIGINAL ARTICLE

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Predominant Hepatitis B virus genotype in association with clinical complictions observed among Bangladeshi chronic carriers

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ABSTRACT

Background: *Hepatitis B virus (HBV) infection is one of the major causes of chronic liver diseases, including liver cirrhosis (LC) and hepatocellular carcinoma (HCC) and affects over 240 million people worldwide. HBV is frequently variable and categorized into ten genotypes (A-J) based on nucleotide divergence of more than 8% of the complete genome which is important for clinical outcomes and disease progressions.* ***Objectives:*** *To explore the prevalence of Hepatitis B virus genotype among Bangladeshi chronic carrier and their association with the clinical complications.* ***Methods:*** *A small cohort was performed between March 2014 and October 2016 with 172 HBV DNA positive patients from the Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh. From them, 29 HBV DNA samples were isolated for sequencing by Sanger method. HBV genotype was determined by phylogenetic analysis of the 750 nucleotide fragment from the Polymerase gene.* ***Results:*** *The results of our study showed genotype C in 17 (58.6%) HBV isolates, genotype D in 10 (34.5%) and genotype A in 2 (6.9%).* ***Conclusion:*** *We conclude that genotype C is predominant in Bangladesh. This is followed by genotype D, and genotype A is the least dominant. Genotype C and A strains are found to be related with more complication. Therefore, patients infected with these HBV strains need to be monitored carefully to assess their clinical outcomes in future.*

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INTRODUCTION

Hepatitis B virus (HBV) is a DNA virus of the Hepadnaviridae family that infects hepatocytes and causes infectious hepatitis. HBV show a variety of clinical pictures ranging from asymptomatic infection to complete resolution or acute fulminant or chronic hepatitis that may lead to life threatening conditions, such as, liver cirrhosis and hepatocellular carcinoma (HCC).[1] Globally, HBV poses a major public health hazard where more than 240 million people are chronically infected with this virus.[2] The highest prevalence of HBV is found in Sub-Saharan Africa and South-East Asia. In the Indian subcontinent

and the Middle East region, about 2-5% population are chronically infected with this virus.[2] Bangladesh belongs to the intermediate prevalence region for HBV infection.[3] About 5%-6% of apparently healthy individuals are HBV carriers in Bangladesh and most of them are unaware of their disease.[4-6] About 6-8 million populations are chronically infected with HBV and most of them are younger.[7,8] The lifetime risk of acquiring HBV is from 20% to 60%.[9] Various studies have shown that HBV is responsible for 31.25% cases of acute hepatitis and 76.3% cases of chronic hepatitis, 61.15% cases of cirrhosis of liver and 33.3% cases of HCC in Bangladesh.[3,10,11]

Many HBV genotypes, sub-genotypes, different HBV genomic mutants and recombinants emerge over time because the reverse transcriptase (RT) of HBV does not have proof reading ability. HBV is classified into ten genotypes (A to J) according to 8% of its genomic divergence.[12] About 4-8% nucleotide sequence variation is required for subgenotyping of HBV.[13] There are distinct geographical distribution of different genotypes with important roles for tracing the evolution and transmission of the virus. Different genotypes play important roles for the course, severity and complications of disease and treatment responses.[14,15] The HBV genotypes appear to influence not only the natural history of HBV related liver disease but also the response to HBV treatment. Genotype A has a tendency for chronicity, While, viral mutations are frequently encountered in genotype C. Both chronicity and mutation frequency are common in genotype D. HBV genotypes are also linked with both core promoter and basal core promoter (BCP) mutations. Genotypes A and B appear to have higher rates of spontaneous HBeAg seroconversion. More advanced liver disease and progression to HCC is more often seen in chronic infection with genotypes C and D, in contrast to genotypes A and B. More specifically, sub-genotypes A1, C, B2-B5 and H appear to be associated with more serious complications than genotypes A2, B1 and B6. Genotypes A and B have higher response rates to interferon based therapy than genotypes C and D.[16] Therefore, knowledge of HBV genotypes enable to clinicians to identify patients at increased risk of disease progression whilst aiding the selection of appropriate antiviral therapy. Genotyping and subtyping can also provide useful information for epidemiological studies.[13] Bangladesh is a country with intermediate HBV carrier prevalence; however little is known about the incidence of HBV genotypes in circulation. Therefore, we investigated the HBV genotype distribution in Bangladesh and then observed its clinical complications.

METHODS

Patients

This small Bangladeshi cohort was done in between March 2014 and October 2016 with chronic hepatitis B (CHB) patients who came to the department of Virology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh for their HBV DNA routine laboratory tests. The basis of selection criteria were HBsAg positivity for >6 months and HBV DNA positive detected by real-time PCR. A total of 172 HBV DNA positive CHB patients were interviewed at BSMMU, who were native residents of different cities of Bangladesh. From them, a total of 29 patients were randomly selected in this study. Blood samples were collected into a 5% EDTA containing microcentrifuge tube. The plasma samples were separated by centrifugation and stored at -20°C until analysis. This study had ethically approved from the Institutional Review Board of BSMMU and written informed consent was obtained from all study patients.

HBV DNA preparation and amplification

Viral DNA was extracted from 200 µl of plasma by D Neasy Blood and Tissue Kit (QIAGEN, Venlo, Limburg, The Netherlands). A nested PCR protocol was followed to amplify 1014-bp region of the partial *Pol* gene of HBV DNA and the primers were: 3079-3099 (5′- AGC CCT CAG GCT CAG GGC ATA-3′) / 1163-1140 (5′- CGT TGC CKD GCA ACS GGG TAA AGG-3′) as external primers and 3192-

3211 (5′- TCA TCC TCA GGC CAT GCA GT-3′) / 991-972 (5′-GAC ACA CTT TCC AAT CAA TNG G-3′) as internal primers (Biobasic, Canada). Finally, 1014-bp fragment of HBV DNA was obtained and detected by ethidium bromide staining in an agarose gel.

HBV DNA purification and sequencing

Nested PCR Products were cleaned by ExoSAP-IT (USB Corp, Cambridge). Internal primers and BigDye®Terminator v3.1 Cycle Sequencing Kit (California) were used for cycle sequencing. The products from cycle sequencing reactions were purified by BigDye® XTerminator™ Purification Kit and sequenced by an automatic sequencer (ABI PRISM® 3500xL Genetic Analyzer).

Phylogenetic analysis and HBV genotyping:

For sequence alignment as well as phylogenetic analysis, we selected the GenBank sequences with the best and the high scoring matches with our sequences in a NCBI BLAST search. Sequences were edited using Chromas 2.3 (Technelysium) and pairwise aligned by ClustalW program. The750 nucleotide sequences in the partial *pol* gene of HBV were analyzed using BioEdit 7.0.9.0 suite of programs.[17] Genotyping was done by phylogenetic analysis. Phylogenetic trees were constructed by the neighbor joining (NJ) method by MEGA 6.06 package.[18,19] Final dataset contains 250 amino acid positions. Genotyping was also determined by using the three online tools.[20] Statistical analysis was done by SPSS software package 17.0 (IBM SPSS Statistics for Windows, Armonk, NY, USA).

RESULTS

A total of 29 patients were selected for isolation and sequence analysis of HBV DNA. Among them, 25 (86.2%) patients were male and 4 (13.8%) patients were female with a mean age of 29.8±12 years and age range of 4 to 50 years. According to the treatment history of the study patients, 15/29 (51.7%) patients untreated and n-14 (48.3%) patients were treated with antiviral drugs. Their viral load values varied from 9.1×10^{2} to 7.0×10^{8} IU/ml, with mean value of 5.5×10^{7} (SD±1.5×10^{8}) IU/ml. ALT values varied from 15 to 419 U/I, and mean ALT level was 89.8 (SD±74.9) U/I. Among the study population, 22 (75.9%) patients were HBeAg positive and 7 (24.1%) patients were HBeAg negative.

Phylogenetic analysis of 250 aa sequences in the *Pol* protein of revealed that only three major HBV genotypes were detected in this study, genotype C was detected in 17 (58.6%) patients, genotype D was detected in 10 (34.5%) patients, while genotype A was detected in only 2 (6.9%) patients (Figure 1).

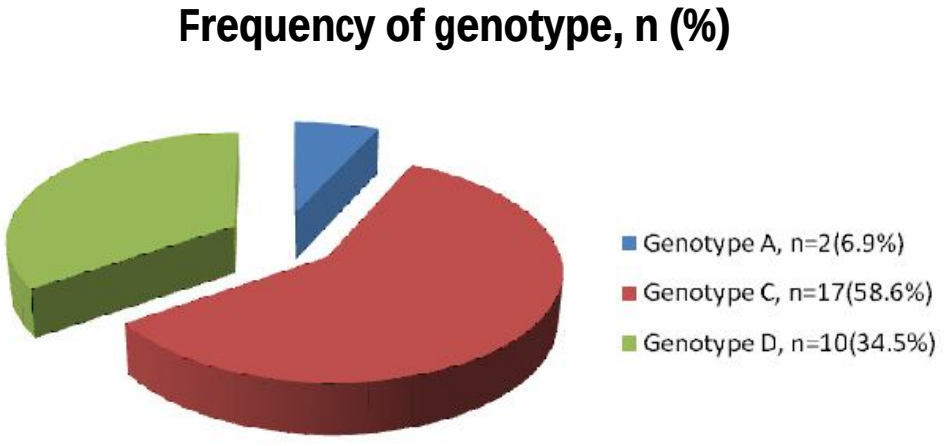

Figure 1: Frequency of genotype, n (%)

Present study showed that the mean HBV viral load of genotype C of HBV positive patients was $6.2\times10^7\pm1.7\times10^8$ IU/ml with mean ALT level was 102.2±94.1 U/l. Whereas, the mean HBV viral load of genotype D of HBV positive patients was $6.1\times10^7\pm1.4\times10^8$ IU/ml with mean ALT level was 76.9±27.1 U/l (Table I). In addition, mean viral load of two patients infected with genotype A strains was $8.1\times10^5\pm9.6\times10^5$ IU/ml with mean ALT level was 50±36.8 U/l. After observation of the study patients from March 2014 to October 2016, we observed that HCC was developed in 2 patients infected with genotype C strains. Both of the genotype A (adw2) positive patients died: one patient from HCC and another patient by LC (Table I).

Table I: Laboratory data and observed clinical complications of Chronic Hepatitis B patients infected with different genotypic strains of HBV

Genotypes of HBV (2014)	Viral load, Mean±SD (IU/ml) (2014)	ALT, Mean±SD (U/l) (2014)	Observed Clinical complications = Number of patients (2014-2016)
Genotype C	$6.2\times10^7\pm1.7\times10^8$	102.2±94.1	HCC=2
Genotype D	$6.1\times10^7\pm1.4\times10^8$	76.9±27.1	ND
Genotype A	$8.1\times10^5\pm9.6\times10^5$	50±36.8	HCC=1 LC=1

Note: HBV viral load in plasma; ND: not ditected; HCC: Hepatocellular Carcinoma; LC: Liver Cirrhosis.

DISCUSSION

The extent of HBV replication among Chronic Hepatitis B (CHB) patients is considerable, reaching $>10^8$ to 10^{11} viral particles per day.[21] As *Pol* is a Reverse transcriptase (RT) that lacks proof reading capacity, HBV replication is also associated with a high mutational rate of 10^5 substitutions /base /cycle.[22] Thus, all possible single base changes in the HBV genome are generated daily, therefore, many genotypes, sub-genotypes and subtypes of HBV and also different type of genomic mutations emerge. The genomic changes associated with antiviral resistance and stable mutations in HBsAg and these resistant viruses are transmitted to other individuals.[23]

On the analysis of our results we found, only three HBV genotypes in the present study, genotype C, genotype D and genotype A. Genotype C (58.6%) was the predominant genotype, followed by genotype D (34.5%), while genotype A was the least dominant genotype observed in only 6.9% of patients. This finding showed similarity with the previous study for HBV genotyping in Bangladesh, the author Rahman et al.[24] reported that the most prevalent genotype was genotype C (48.7%) followed by genotype D (28.2%) and genotype A (23.1%). On the contrary, other two previous Bangladeshi studies found that the prevalent genotype was genotype D, followed by genotype C and genotype A.[25,26] Whereas, the neighbouring country, Eastern India, Kolkata showed presence of genotype C in addition to genotypes A and D among the HBV carriers.[27] Whereas, Western and Northern India showed that genotypes D and A were more prevalent.[28,29] Prevalent genotypes in Srilanka were B (36%), C (16%), D (12%) and in Pakistan were genotypes D (91.1%) and C (9.8%).[30,31]

Earlier studies suggested that HBV genotype C is associated with delayed HBeAg seroconversion, more active hepatitis, lower response to antiviral therapy, more advanced liver disease and a higher risk of hepatocellular carcinoma.[32-35] On observation of our present study showed most of the genotype C positive patients were associated with elevated ALT that indirectly reflects more liver damage. On further observation, we found that two of our patients infected with genotype C

strains had died due to HCC. Interestingly, we found that only two patients were infected with genotype A strain and both patients died: one patient due to HCC and another patient by LC.

Limitations

The present study was based on a small cohort due to limitation of fund, thus large sample size is needed to confirm the validity of these findings.

CONCLUSION

In conclusion, the most prevalent HBV genotype is genotype C, followed by genotype D, while genotype A is the least dominant among Bangladeshi CHB patients. Genotype C and A strains are found to be related with more complications, therefore, patients infected with these HBV strains need to be monitored carefully to assess their clinical outcome in future.

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Ultrasonographic Measurement of Renal Length in Chronic Kidney Disease with eGFR Correlation

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ABSTRACT

Introduction: *Chronic kidney disease (CKD) is global health problem causing significant mortality and morbidity. This study was carried out to determine the correlation of ultrasonographically measured renal length with estimated glomerular filtration rate (eGFR) in patients with CKD.* ***Methods:*** *This cross-sectional study was carried out in the department of Radiology and Imaging, Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM) during the period of July 2016 to June 2017, enrolling 42 patients having clinical diagnosis of CKD who were not on dialysis and who have at least three serum creatinine reports records within 90 days of the ultrasound. All the patients underwent ultrasound examination to measure the renal length of both kidneys. The renal ultrasound examinations were done by the researcher at first, and then confirmed by a consultant of the Department of Radiology and Imaging. All findings of serum creatinine, eGFR and ultrasonography were collected in a pre-designed data collection sheet.* ***Results:*** *Significant positive correlation was found between mean renal length and Cockcroft-Gault (CG) eGFR of patients with clinical diagnosis of CKD. The values of Pearson's correlation coefficient was 0.340, which is significant ($p<0.05$).* ***Conclusion:*** *This current study concluded that renal length had weak positive correlation with eGFR. Renal length measured at ultrasound appears to be related to the degree of renal impairment in patients with CKD, who are not on dialysis and routine measurement reporting of renal length should be considered in such patients.*

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INTRODUCTION

Chronic kidney disease (CKD) is a worldwide public health problem. Kidney damage is defined as pathological abnormalities in blood or urine tests or imaging studies. The incidence and prevalence of kidney failure are rising, the outcomes are poor, and the costs of management are high.[1,2] The incidence, prevalence, mortality and cost for patients with kidney failure treated by dialysis and transplantation, the end stage of CKD, have increased steadily during the past two decades. The major outcomes of CKD, regardless of cause, include progression of kidney failure, complications of decreased kidney function, and cardiovascular disease (CVD). Increasing evidence indicates that some of these adverse outcomes can be prevented or delayed by early detection and treatment.[3] Glomerular filtration rate (GFR) is the best measure of overall kidney function in health and disease. The GFR represents the rate at which an ultrafiltrate of the plasma is formed by the glomeruli. Normal GFR in young adults is approximately 120 to 130 mL/min per 1.73 m^2 and declines with age.[4] A GFR level less than 60 mL/min per 1.73 m^2 represents loss of half or more of the adult level of normal kidney function. Below this level, the prevalence of complications of chronic kidney disease.[5] Renal function is commonly assessed by serum creatinine (S_{cr}), but it has some shortcomings. Both the muscle mass and quantity of ingested meat in addition to the urinary clearance will determine the level of the serum creatinine. Some drugs, including trimethoprim and cimetidine, inhibit creatinine secretion, thereby reducing creatinine clearance and elevating the serum creatinine level without affecting the GFR. S_{cr} may remain within the normal range despite a reduction in GFR of 60% or greater. So the use of S_{cr} alone as a measure of renal function is not reliable.[6] Also blood urea nitrogen (BUN) is a poor marker of GFR as it is heavily influenced by state of hydration, nitrogen load and metabolism.[4,6] Chronic kidney disease is defined as either kidney damage or GFR < 60 ml/min/1.73$m^2$for $\geq$ 3 months.[7]

Ultrasonography is one of the several methods to evaluate renal morphology. Different studies showed that ultrasonography is a rapid and non-invasive diagnostic method for renal disease and also the first method of choice for screening and follow up of patients and healthy people.[7] Traditional technique is that renal length correlates with renal function in CKD, and therefore, bipolar renal lengths are almost always reported at renal ultrasound.[8]

The purpose of this study was to determine whether there is a relationship of renal length measured at ultrasound with estimated glomerular filtration rate (eGFR) as renal function in patients with CKD, using two widely accepted computational methods of estimating GFR. This study was directed towards looking for a simpler method of determination of functional capacity of kidneys in CKD, particularly in resource poor settings. So, this study was supposed to help evaluating the relationship of estimated glomerular filtration rate (eGFR) with renal length in CKD by ultrasonography which is easily available, less costly, radiation free and non-invasive.

METHODS

This cross-sectional study was carried out in the department of Radiology and Imaging, Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM) during the period of July 2016 to June 2017. A total of 52 patients having the clinical diagnosis of CKD, who were not on dialysis, at least three serum creatinine reports and weight recorded within 90 days of the ultrasound, were selected for ultrasound scanning. A total of 10 subjects were excluded due to sonographic findings of hydronephrosis and 42 patients were finally enrolled in this study. Prior to the commencement of the study, the research protocol was approved by thesis committee (Local ethical committee). The objectives of the study along with its procedures, risks and benefits of this study were explained to the subjects in easily understandable local language and then informed written consent was taken from each subject. It was assured that all information's and records would be kept confidential and the procedure would be helpful for both physicians and the patient for the evaluation of the functional capacity

of kidneys in CKD, by ultrasonography which is easily available, less costly, radiation free and non-invasive. Patients on dialysis were not included in the study. A detailed history and physical examination with emphasis on the urinary system was recorded. The lowest creatinine performed within 90 days of the ultrasound was used for estimated glomerular filtration rate (eGFR) calculations, as it represents the best recorded renal function during the study period and helps to minimize the influence of superimposed acute on chronic renal insufficiency.[4] The Cockcroft-Gault (CG) and the modification of Diet in Renal Disease Study (MDRD) equations were used for estimated glomerular filtration rate (eGFR) calculation.[1] All the patients underwent ultrasound examination to measure the renal length of both kidneys. The renal ultrasound examinations were done by the researcher at first, and then confirmed by a consultant of the Department of Radiology and Imaging. All findings of serum creatinine, eGFR and ultrasonography were collected in a pre-designed data collection sheet. Statistical analysis of the results was done by computer software devised as the statistical package for the social science (SPSS-20). The results were presented in tables, figures and diagrams. Mean renal length was used in analysis. The relationship between ultrasound measurements and estimated glomerular filtration rate were tested using Pearson's correlation coefficient test and linear regression. Significance was considered at a '*p*' value <0.05.

RESULTS

The age of the patients ranged from 42 to 85 years and the maximum number was found in 7th decade. The mean (±SD) age was 65.3 years with standard deviation ±10.4 years (Table I).

Table I: Age distribution of the study patients (n-42)

Age (in year)	No. of patients	Percentage
≤50	3	7.1
51-60	10	23.8
61-70	18	42.9
71-80	7	16.7
>80	4	9.5

Mean (±SD) weight was 63.1±6.9 kg with range from 55 to 85 kg (Table II).

Table II: Weight distribution of the study patients (n-42)

Weight (in kg)	No. of patients	Percentage
51-60	18	42.9
61-70	19	45.2
>70	05	11.9

The CG eGFR of the study patients were considered and it was found that maximum (25 patients, 59.5%) patients' CG eGFR belonged to 30-59 ml/min. The mean (±SD) CG eGFR was 34.3± 14.0 ml/min with range from 9.0 to 65.1 ml/min (Table III).

Table III: Distribution of the study patients according to the CG eGFR (n-42)

CG eGFR	No. of patients	Percentage
≤50	03	7.1
51-60	10	23.8
61-70	18	42.9
71-80	07	16.7
>80	04	9.5

It was observed that maximum (25 patients, 59.5%) patients had MDRD eGFR within 30-59 ml/min/1.73m^2. The mean (±SD) MDRD eGFR was found 36.3±14.6 ml/min/1.73m^2 with range from 11.0 to 60.9 ml/min/1.73m^2 (Table IV).

Table IV: Distribution of the study patients according to MDRD eGFR (n-42)

CG eGFR	No. of patients	Percentage
<15	02	4.76
15-29	13	30.96
30-59	25	5.52
60-89	02	4.76

The mean renal length on the right side was 9.72±0.91 cm with range from 8.1 to 11.6 cm and the mean renal length on the left side was 10.05±0.97 cm with range from 8.1 to 12.5 cm. The mean renal length was 9.9±0.9 cm with range from 8.1 to 11.9 cm. Mean renal length had significant relation with eGFR (Table V).

Table V: Mean renal length of the study patients (n-42)

Mean renal length in cm	Mean±SD	Range (min-max)
Right side	9.72±0.91	8.1-11.6
Left side	10.05±0.97	8.1-12.5
Both sides	9.88±0.91	8.15-11.85

Table VI: Pearson's correlation coefficient of different parameters of the study patients

Relation	r value	*p* value
Mean renal length vs CG eGFR	0.810	0.028[s]
Mean renal length vs MDRD eGFR	0.317	0.033[s]

s-significant

DISCUSSION

Renal length has traditionally been considered a surrogate marker of renal function, because renal length decreases with decreasing renal function. Renal lengths are universally reported and are usually the only measurements given at renal ultrasound.[8] In this current study, it was observed that the mean (+SD) age was 65.3 years with standard deviation + 10.4 years with range from 42 to 85 years and most (42.9%) of the patients with chronic kidney disease was found in 7th decade. Poggio et al.[9] found the mean +SD age 56+16 years with range from 34 to 76 years which is comparable with the current study. Levey et al.[10] showed that the mean +SD age was 50.6+12.7 years. Moghazi et al.[11] and Rule et al.[12] observed that the mean age was 45 years with range from 15-82 years and 41+11 years with range from 18-72 years respectively. Adibi et al.[13] have showed that the mean+SD age 38.8+7.7 years with range from 20 to 50 years, where the current study was higher with the above mentioned studies. In this present study it was observed that 52.4% and 47.6% were male and female respectively and male female ratio was almost 1, 1:1, which is similar to the study of Beland et al.[14] and Moghazi et al.[11]

In this current series, mean (±SD) weight was 63.1±6.9 kg with range from 55 to 85 kg and maximum (45.2%) patients were 61-70 kg in weight. Rule et al.[12] and Adibi et al.[13] have observed higher mean weight, which was 82±18 kg with range from 47-162 kg and 71.3±12.8kg with range from 36 to 120 kg respectively. Similarly, Poggio et al.[9] showed that the mean weight was 81.0±20.3 kg with range from 57.0 to 109.0 kg, which may be due to their higher body surface area in their study patients.

In this current series, it was observed that the mean (±SD) CG eGFR was 34.3±14.0 ml/min with range from 9.0 to 65.1 ml/min and maximum (59.5%) patients had eGFR within 30-59 ml/min, followed by 31.0% or eGFR of patients within 15-29 ml/min, 4.8% patient's eGFR <15 ml/min or dialysis and 4.8% patient's eGFR within 60-89 ml/min. Similarly, the mean (±SD) MDRD eGFR was found 36.3±14.6 ml/min/1.73m^2 with range from 11.0 to 60.9 ml/min/1.73m^2 and maximum (59.5%) patients had moderately decreased GFR (30-59 ml/min/1.73m^2), followed by 26.2% severely decreased GFR (15-29) ml/min/1.73m^2), 7.1% kidney failure (<15 ml/min/1.73m^2 or dialysis) and 7.1% kidney damage with mild decreased GFR (60-89 ml/min/1.73m^2). Similarly, Beland et al.[14] found the mean eGFR using CG was 34.8 ml/min with range from 10.6-99.4 ml/min and 36 ml/min with range from 8-66 ml/min using MDRD, which is closely resembled with the current study. In another study, Poggio et al.[9] showed that the mean±SD CG GFR was 31±26 ml/min with range from 10-70 ml/min. Similarly, mean±SD MDRD GFR was 37±30 ml/min/1.73m^2 with the range from 12 to 80 ml/min/1.73m^2, which is comparable with the present study. Levey et al.[10] found most (45.9%) patients had moderately decreased GFR (30-59 ml/min) fol-

lowed by 28.6% severely decreased GFR (15-29 ml/min), 13.9% kidney damage with mild decreased GFR (60-89 ml/min), 9.6% kidney failure (<15 or dialysis) and 2.0% kidney damage with normal or increased GFR (>90 ml/min), which is closely resembled with the current study.

In the current study, mean renal length on the right side was 9.72±0.91 cm with range from 8.1 to 11.6 cm, on the left side was 10.05±0.97 cm with range from 8.1 to 12.5 cm and mean renal length (both right and left) was 9.9±0.9 cm with range from 8.15 to 11.9 cm. Beland et al.[14] found that the mean renal length was 10 cm with range from 7.2-12.4 cm. In another study done by Adibi et al.[13] showed the mean length of kidneys was 10.22 cm with 95% CI: 10.11-10.34 cm. The mean length was observed by the author was 10.25 cm with 95% CI: 10.11-10.40 cm and 10.19 with 95% CI: 10.07-10.32 cm for the left and right kidneys, respectively, which was not significant (p> 0.05). Sanusi et al.[15] showed the right renal length ranged between 7.86 to 12.18 cm with a mean±SD of 10.34±1.28 cm and the left kidney length range was 7.0 to 13.0 cm with a mean±SD of 10.33±1.50 cm. The values of renal length of right side and left side obtained in the present study strongly support these investigators. This present study showed that renal length had positive correlation with eGFR. Correlation coefficient of renal length with CG eGFR was r=0.340, (p<0.05) and with MDRD eGFR was r=0.317 (p<0.05), which were correlated with eGFR. So, Ultrasonographycally measured renal length correlates with estimated Glomerular Filtration Rate (eGFR) in patients with chronic kidney disease. In another study, Adibi et al.[16] showed a correlation between GFR and ultrasonographic kidney sizes, especially the kidney length.

CONCLUSION

From the current study, it is concluded that renal length had strong positive correlation with eGFR. Renal length measured at ultrasound appears to relate to the degree of renal impairment in patients with chronic kidney disease, who are not on dialysis, and routine measurement reporting of renal cortical thickness should be considered in such patients.

Conflict of interest: There is no conflict of interest.

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CASE REPORT
NORTH BENGAL MEDICAL COLLEGE JOURNAL [2020], VOL., 6, No. 2 : 33-36

A 14-years-old girl with Sterile Pyuria due to Renal Tuberculosis: A Case Report

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ABSTRACT

A 14-years-old girl presented with a 2-years history of a dull ache in her right flank along with recurrent urinary tract infection and weight loss of 10 kg over two years. She was treated in upazila health complex and local health care centres without any improvement. Then she was admitted in medicine ward of North Bengal Medical college, Sirajganj, Bangladesh. There was no history suggestive of tuberculosis and examination was unremarkable except for cachexia and a tender, palpable right kidney and tender right renal angle. Investigations including complete blood count showed Hb-9 gm/dl and ESR 90 in 1st hour. Tuberculin skin test showed induration 18 mm at 72 hours and X-ray showed dense opacity in right renal area. Ultrasonography of the abdomen revealed right sided hydronephrosis, CT urogram showed right renal and ureteric calcification with hydronephrosis. Urinary sample for polymerase chain-reaction (PCR) assay for Mycobacterium tuberculosis was positive. She started anti tuberculous chemotherapy and responded well.

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INTRODUCTION

Tuberculosis is a disease caused by *Mycobacterium tuberculosis* and pulmonary manifestation is most common.[1] In other extra pulmonary cases, tuberculous lymphadenitis is frequent, followed by urogenital tuberculosis, which represents about 30% of the extra pulmonary cases worldwide[2,3] and reaches rates of 40–60% in developed countries.[4] Tubercle bacillus infection is almost always airborne and it is followed by replication of the bacillus in alveolar macrophages which form the characteristic Ghon focus.

After a latent infection renal TB may be due either to a disseminated infection or to a primitive genitourinary localization. The majority of patients have pyuria, sometimes with hematuria. The diagnosis of urinary tuberculosis is based on the finding of pyuria in the absence of infection by common bacteria. Although sterile pyuria strongly suggests that genitourinary tuberculosis, but it should be investigated.[2-5] Secondary bacterial infections may occur in up to 50% of the cases[4] and patients with immunosuppression like HIV infection, diabetes are at higher risk.[2]

The Case

A 14-years-old girl presented with a 2-years history of a dull ache in her right flank along with recurrent urinary tract infection and weight loss of 10 kg over two years. There was no history suggestive of tuberculosis. There were no exacerbating or relieving factors. The pain did not radiate. There was no history of fever, chronic cough, anorexia and hematuria or dysuria. She did not have any bowel symptoms. There was no family history of tuberculosis. She was vaccinated as per EPI schedule. On examination the only finding was a tender and palpable right kidney and tender right renal angle. Chest examination was unremarkable. Investigations including complete blood count showed Hb-9 gm/dl, total leucocyte count was 8,600/mm3 of blood with a lymphocyte count of 25%. Her serum creatinine was 1.1 mg/dl, ESR 90 in 1st hour and blood urea was 30 mg/dl. Tuberculin skin test showed induration 18 mm at 72 hours and Chest x-ray (P/A) was normal. Abdominal X-ray showed dense opacity in the right renal area. Ultrasonography of the abdomen revealed right sided hydronephrosis, CT urogram showed right renal and ureteric calcification with hydronephrosis (Figures 1, 2). Blood and urine examination, the letter with culture, were requested. The absence of bacterial growth along with leukocyturia led to the investigation for the acid-alcohol resistant bacillus. Early in the morning three urine samples were collected and a urinary sample for Gene Xpert/RIF polymerase chain-reaction assay for *Mycobacterium tuberculosis* was positive with Rifampicin sensitivity (Figure 3). Culture for mycobacterium was not done.

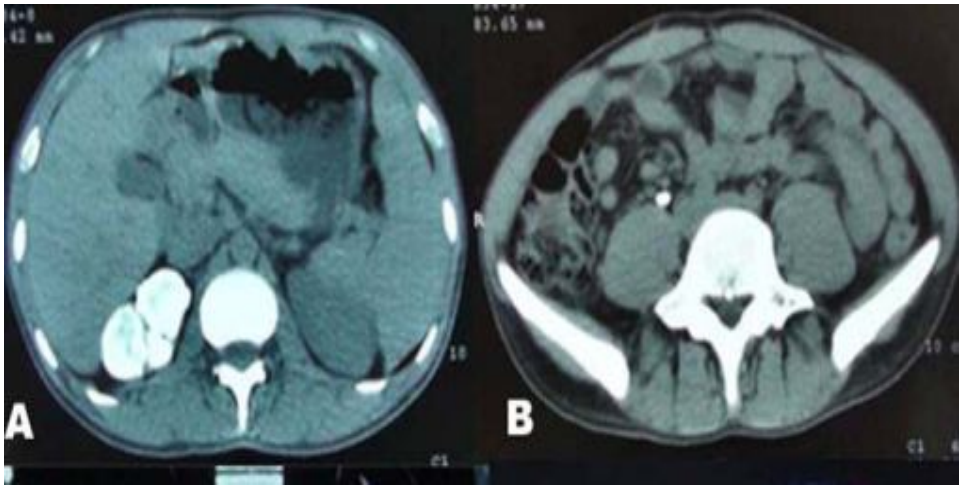


Figure 1: (A) Axial non-contrast CT image showing renal calcification
(B) Non-contrast axial cut showing ureteral calcification

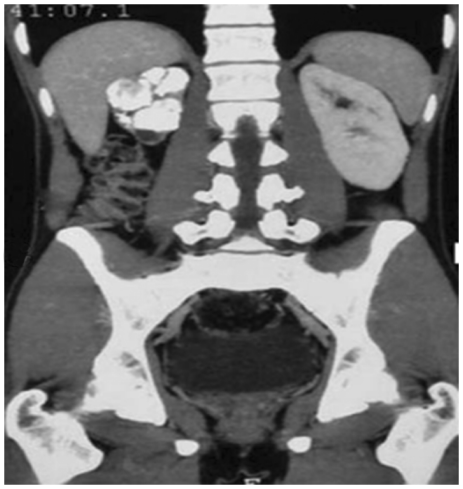
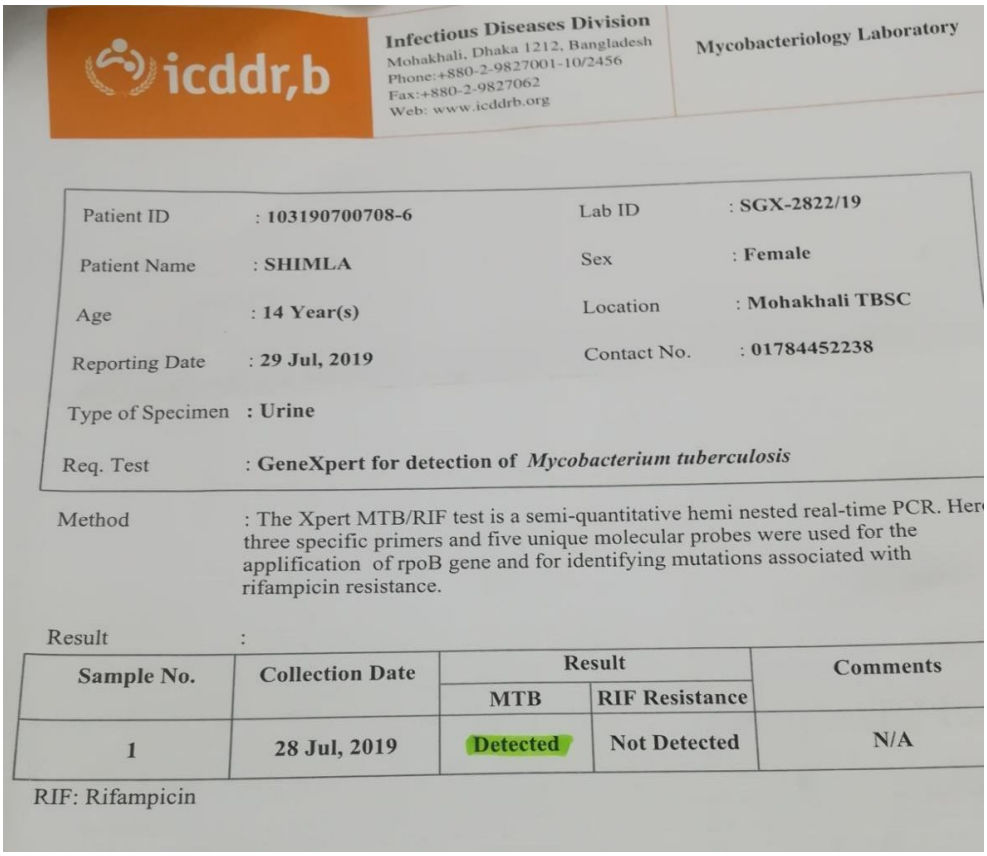


Figure 2: Reconstructed image showing right renal calcification



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Mycobacteriology Laboratory

Patient ID	: 103190700708-6	Lab ID	: SGX-2822/19
Patient Name	: SHIMLA	Sex	: Female
Age	: 14 Year(s)	Location	: Mohakhali TBSC
Reporting Date	: 29 Jul, 2019	Contact No.	: 01784452238
Type of Specimen	: Urine		
Req. Test	: GeneXpert for detection of *Mycobacterium tuberculosis*		

Method : The Xpert MTB/RIF test is a semi-quantitative hemi nested real-time PCR. Her three specific primers and five unique molecular probes were used for the applification of rpoB gene and for identifying mutations associated with rifampicin resistance.

Result :

Sample No.	Collection Date	Result		Comments
		MTB	RIF Resistance	
1	28 Jul, 2019	Detected	Not Detected	N/A

RIF: Rifampicin

Figure 3: Gene X pert /RIF polymerase chain-reaction assay for Mycobacterium tuberculosis

She started anti tuberculous chemotherapy with rifampicin, isoniazid and pyrazinamide on 31/07/2019 and then responding well with resolution of symptoms and weight gain of 36 kg from 28 kg. After anti tuberculous chemotherapy for 12 months, it was decided to refer the patient to urosurgical evaluation.

DISCUSSION

Tuberculosis is due to the *Mycobacterium tuberculosis*, which is usually very resistant to environmental factors and forms a specific type of granulomatous inflammation called caseous granuloma. *M. tuberculosis* can enter the body through mucous membranes or through the skin.[2,3] Development of tuberculosis is dependenton the patient's immune response to the infection, route of infection and lymphatic or haematogenous spread of the organism. In granulomatous lesions cell-mediated immunity effectively regulates bacterial restraint and in most cases mycobacterium persist in a latent state as eradication is not entirely possible.[4]

Renal infection, usually following the haematogenous spread of the mycobacterium, is characterized by the formation of micro-abscesses around the periglomerular capillary. The immune system is usually capable of blocking the disease with the formation of small inactive granulomas. In advanced stages of granulomas may coalesce with the formation of cavities, later may communicate with the calyces, and thus allow the infection of the downstream anatomical structures such as the renal pelvis, ureter, bladder and urethra. A further destruction of the kidney then takes place with the formation of multiple scars and deformed excretory system filled with caseous material resulting in pyonephrosis, ultimately resulting in the formation of fibrosis of kidney known as chalk or putty kidney.[6,7]

Symptoms of renal tuberculosis resemble symptoms of low urinary tract infection. One has to suspect when conventional antimicrobial therapy is not effective to eradicate infection or there is presence of sterile pyuria,[3] a fact that often re-

sults in late disease diagnosis. The cytological analysis of urine in specific colouration is sensitive to detect mycobacteria, like *Mycobacterium bovis* or *Mycobacterium avium-intracellulare* complex, but very little specifically for *M. tuberculosis. S*pecific culture for *M. tuberculosis* or through polymerase chain reaction (sensitivity=94.7%) will confirm *M. tuberculosis*.[4,5,8-10]
Calcifications are common and easily identified in a simple abdominal x-ray. Abdominal CT scan is mandatory to observe the structural changes and renal parenchymal calcification.[9,11]
The radiological alterations based on different presentations seen in ultrasound of renal system can be classified into six different types: nephrectasia (type I), hydrops (type II), empyema (type III), calcification (type IV), inflammatory and atrophic (type V) and mixed (type VI).[12]
A series of 102 cases has already shown the importance of early diagnosis and proper treatment of renal tuberculosis.[13] The treatment resides in the administration of early introduction of anti tuberculous chemotherapy and or partial or total excision if structural abnormalities are present. We use pyrazinamide-rifampicin isoniazid based regimen which is renal friendly. Precautions required regarding use of streptomycin, ethambutol and other aminoglycosides as these are nephrotoxic, so dose adjustment is recommended.[2] Failure to early detection and treatment results unfortunate and extreme adverse outcomes.

Conflict of interest: None

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CASE REPORT
NORTH BENGAL MEDICAL COLLEGE JOURNAL [2020], VOL., 6, No. 2 : 37-41

Mucoepidermoid Tumor of Parotid Gland with Multiple distant Metastases: A Case Report

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ABSTRACT

Tumours of salivary glands are uncommon and comprise of about 2%–4% of all head and neck tumours. About 75%–80% of these tumours are benign and include pleomorphic adenoma, monomorphic adenoma, oncocytoma and papillary cystadenoma lymphomatosum. Mucoepidermoid carcinoma is the most common malignant tumour of salivary glands, representing 5–10% of all salivary gland tumours although known to be metastatic to local lymph nodes, distant metastases are rare (especially, with low and intermediate grade tumours). Histologic grade and the expression of various mucin glycoproteins are useful prognostic indicators. We present a case of mucoepidermoid carcinoma of parotid gland origin with distant metastases which is an uncommon occurrence with intermediate grade tumours.

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INTRODUCTION

Mucoepidermoid carcinoma (MEC), arises from pluripotent reserve cells of excretory ducts that are capable of differentiating into squamous, columnar, and mucous cells.[1,2] Although MEC accounts for <10% of all tumours of the salivary gland, it constitutes approximately 30% of all malignant tumours.[3] Among them, MEC occurs most frequently in the parotid gland. Stewart et al.[4] introduced the term mucoepidermoid to define distinct salivary gland tumour, characterized by a mixed pattern of two main cell types, epidermoid and mucus-producing cells. However, a third cell type, intermediate cell, which is not mucous or fully epidermoid, is often present. Subsequent metastases of few of the previously benign tumours have led to all mucoepidermoid tumours being considered carcinoma.[5] In view of the relative rarity of (salivary / MEC) tumours and remarkable variability in their biological behavior, opinions differ about appropriate classification, grading, and treatment.[6-9] This tumour rarely metastasizes to distant organs. We present a case of parotid gland mucoepidermoid carcinoma with hepatic and brain metastases.

The Case

A 24 year old woman presented with left sided neck swelling with pain in the neck for 6 months. She denied weight loss, fever, chills, dysphagia, dysphonia, facial numbness or paresis. The physi-

cal examination revealed a 4 cm by 3 cm fixed mass with rubbery consistency at the angle of the mandible on left side, without palpable lymphadenopathy. The neurological examination was negative for involvement of the cranial nerves. Initial CT scan of the neck revealed a parotid mass (3 × 2.3 × 2.7 cm) involving the superficial-deep part of the gland with slight displacement of the retromandibular vein and no substantial regional lymphadenopathy. Fine needle aspiration cytology (FNAC) was done from the mass which revealed epidermoid and mucous secreting cells. Subsequently, the patient underwent excision of the involved parotid gland (total parotidectomy). The pathology report was diagnostic of an intermediate grade mucoepidermoid carcinoma of the salivary gland with clear surgical resection margins; the pathological stage was pT2pN0pMx. As the surgical margins were clear, patient was advised regular follow up.

After about one year of follow up, the patient developed headache, a small swelling over left side of vertex and abdominal pain. CT scan of the head and neck with screening of chest and abdomen was done. CT scan of the brain revealed a large heterogeneous extra-axial mass along the left parietal convexity causing partial destruction of left parietal bone, invasion of underlying leptomeninges, left parietal lobe and midline shift towards the contralateral side (Figures 1, 2). CT scan of the neck did not reveal any residual disease in left parotid region but showed multiple metastatic left level II, III and V lymph nodes with cystic changes (Figure 3). CT scan of the chest with screening of abdomen revealed multiple hypodense necrotic nodular enhancing lesions (largest one approximately 62.1 mm × 53.5 mm) in both lobes of liver (Figure 4). CT guided needle biopsy of vertex, hepatic lesions and USG guided FNAC from cervical lymph nodes were histologically positive for malignant cell, favouring metastatic mucoepidermoid tumours.

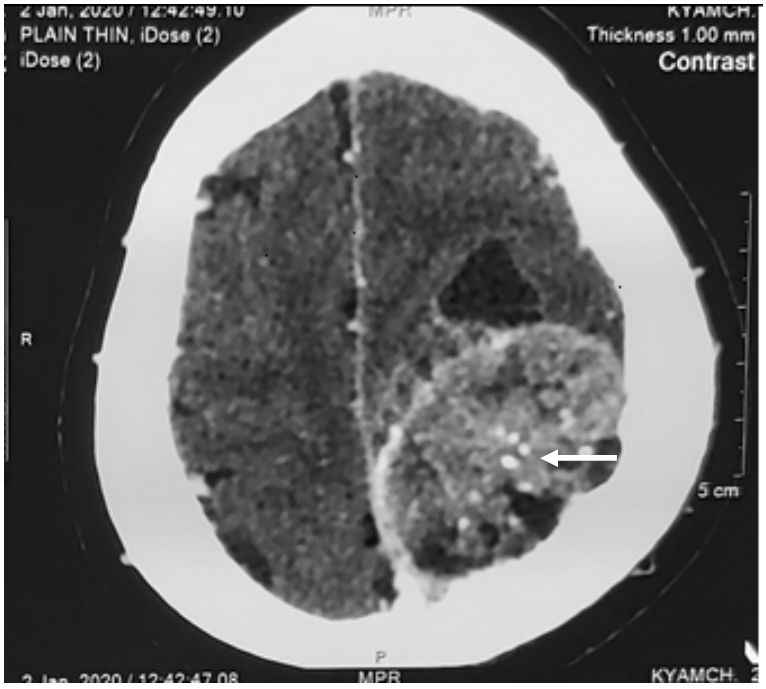

Figure 1: Contrast enhanced CT scan of brain in axial plane showing heterogeneously enhancing lesion with tiny calcifications (arrow) in left posterior parietal lobe causing mid line shift towards contra-lateral side

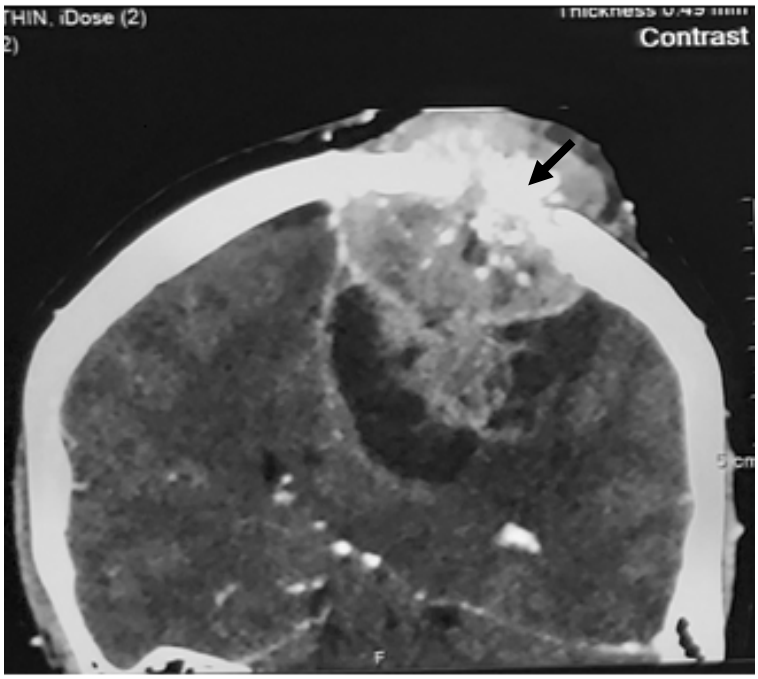

Figure 2: **Contrast enhanced CT scan of brain in reformatted coronal plane showing large heterogeneously enhancing extra-axial mass along left parietal convexity causing partial destruction of left parietal bone (arrow), invasion of underlying leptomeninges, left parietal lobe and mid line shift towards contralateral side**

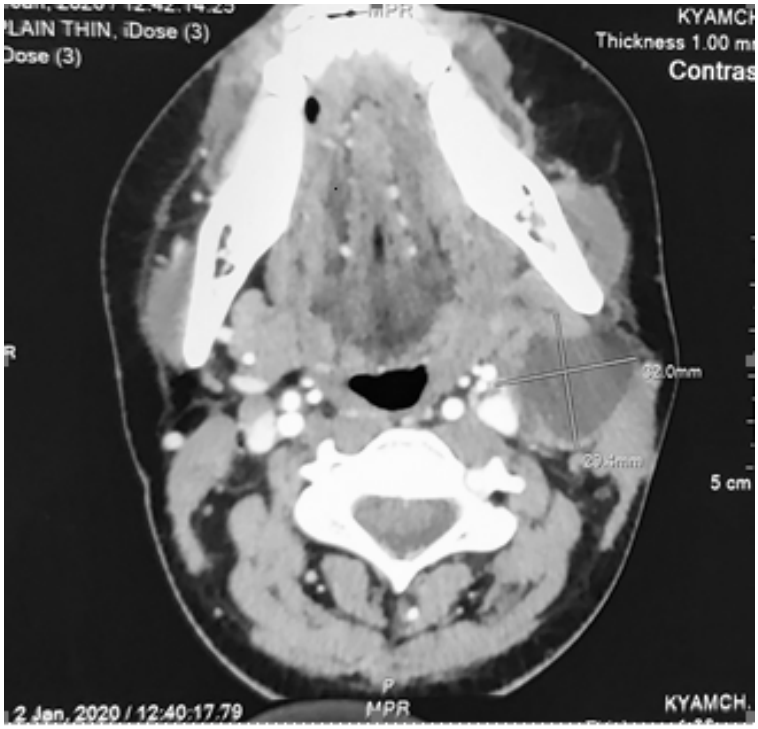

Figure 3: **Contrast CT scan of neck showing metastatic left level II lymph node with cystic change infiltrating adjacent sternocleidomastoid muscle**

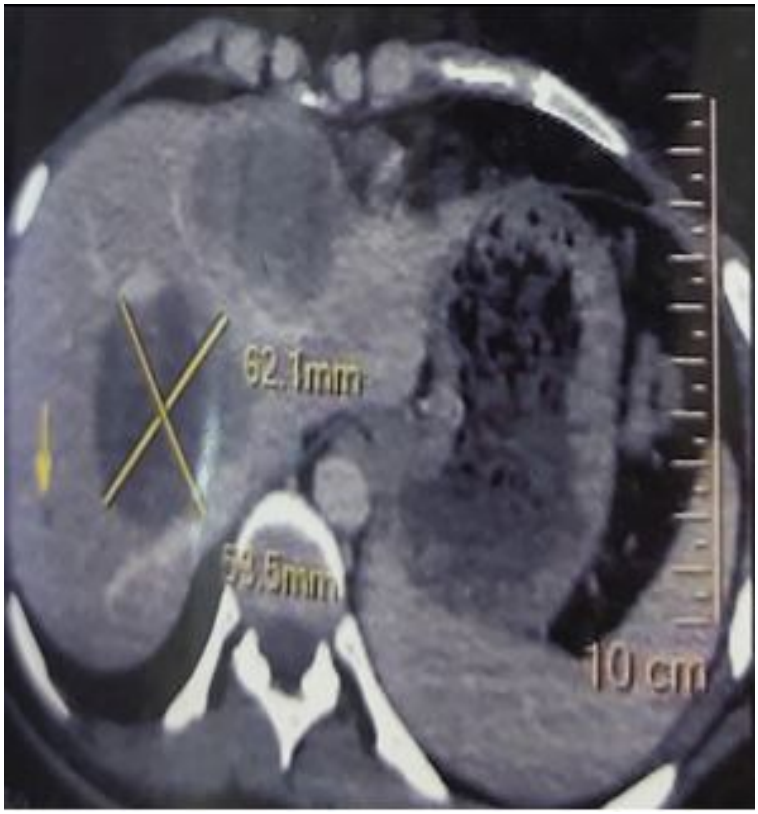

Figure 4: Axial contrast CT scan of abdomen showing hypodense necrotic nodular enhancing lesions within both lobes of liver, largest one at right lobe

DISCUSSION

Mucoepidermoid carcinoma is one of the most common malignant tumours of salivary glands, representing 5%–10% of all salivary gland tumours.[1,2] In our current case, left parotid gland was involved which is comparable with previous studies conducted by Shilo et al.[1] and Batsakis et al.[2] However, these tumours are also known to occur in lips, tongue, and buccal mucosa[1] although known to be metastatic to local lymph nodes, distant metastases are rare (especially, with low and intermediate grade tumours). Our patient had intermediate grade of mucoepidermoid carcinoma, but had multiple sites of distant metastases, which was rare for intermediate variety comparted with previous studies.[3,5,6] The incidence of distant metastasis in head and neck cancer and especially in salivary gland cancer is relatively low in comparison to other malignancies. The presence of distant metastasis has a poor prognosis in head and neck cancer, with a median survival of 4.3-7.3 months.[8] The likelihood of developing distant metastasis is associated with high-grade tumours, such as adenoid cystic carcinoma, salivary duct carcinoma, high-grade mucoepidermoid carcinoma and tumours located in the submandibular gland, posterior tongue and pharyngeal tumours.[2,6] A lower risk of developing distant metastasis is known for all other histological entities of salivary gland tumours. Distant metastases develops in approximately 20% of all patients with salivary gland cancer.[1] The most common site of metastasis is the lung.[8] Liver and brain are very rare sites.[8-10] In the present case, our patient had metastases to regional neck nodes as well as unusual rare site of distant metastases in the brain and liver. However, knowledge of the disease course for distant metastas-

es from salivary gland cancer is limited due to its rarity, the wide variety of salivary cancer histologic subtypes and the often prolonged disease course that could lead to loss of patient follow-up evaluation.[11] Treatment of these patients is usually performed in a palliative setting.

CONCLUSION

Salivary gland cancer exists as 24 different histologic types which can progress in different ways. The most common type is mucoepidermoid carcinoma. The tendency toward distant metastasis varies by primary location. Distant metastasis is less common with tumours that arise in the parotid gland. The current case of mucoepidermoid tumour of parotid gland had distant hepatic and brain metastases during its time-course. So, from this case report, it is advisable to perform metastatic work up when dealing with intermediate grade mucoepidermoid tumour of the salivary gland.

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Conflict of interest: Author declares that there is no conflict of interest.

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Acknowledgments

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