Original Research Article

A study to examine the correlation between nutritional status with bone health of young adult college students of two different communities (Tribal and non-Tribal) by anthropometric measures and urinary indices

ABSTRACT

Aim: The aim of this study was to correlate the nutritional status with bone health of young adult college students of two different communities respectively by anthropometric measures and urinary indices and quantitative ultrasonography.

Study design: Cross-sectional study

Place and Duration of Study: Human Physiology Laboratory, Tripura Institute of Paramedical Sciences, Hapania, Amtali, Tripura (West) 799130, India between October 2011 to March 2013.

Methodology: This study was undertaken in college students of two different communities, Tribal (n=60; male: 30; female 30) and non-Tribal (n=100; male: 50; female: 50), aged between 18 to 21 years. Nutritional status was assessed by measuring height, weight, body mass index (BMI), mid upper arm circumference (MUAC), fat-free mass (FFM), muscle mass (MM) and bone health by measuring skeletal mass (SKM) and urinary indices like calcium, phosphate, creatinine, Ca:Cr ratio and hydroxyproline: creatinine ratio and quantitative ultrasonography (QUS).

Results: Apart from clear observations of significant community and gender variations in anthropometric measurements and indices for assessing nutritional status(MUAC,FFM,MM) and bone health (SKM), prevalence of chronic energy deficiency (CED) was observed more in tribal (25%), than non-tribal (11%) population. A sparse population was observed overweight (tribal 6.67%; non-tribal 8%) and there was no record of obesity. Urinary excretion of markers for bone turnover also revealed significant community and gender variations, and except calcium, no other markers crossed normal reference range. Correlation analyses between anthropometric nutritional markers and urinary bone health markers revealed both positive and negative significant relationships. Regression analyses further revealed strongest association of FFM with SKM explaining 17% to 81% variance. Bone mineral density assessment by QUS diagnosed osteopenia in the studied population irrespective of gender and community.

Conclusion: Results indicate that nutritional status has significant correlation with bone health and nutritional deficiency may cause adverse effect on bone.

Keywords: Nutritional status; bone health; anthropometric measures; urinary markers, community (Tribal and non-Tribal)

1. INTRODUCTION

Several reports have indicated that inadequate food habits along with traditional socio-cultural and biological activities may lead to a high proportion of child as well as adult under-nutrition [10–12]. Earlier, recognizing this issue, National Nutritional Monitoring Bureau (NNMB) of India had undertaken extensive studies on nutritional status of tribal adolescent children during the period from 1998-1999 mainly from nine southern states [11]. Tribe specific similar other studies were also reported from different other states of India like Bihar [13, 14], Orissa [14] and West Bengal. As far as social and population background of the state of Tripura, where this study was undertaken, is one of the seven states of North-East India, where, according to Census of India (2011) and Government of Tripura reported Provisional Population Totals (2011), has a tribal population of 31% [15]. Like all other tribal people of India, tribes of Tripura are also having geographically isolated life-style. However, during the past one or two decades, there is a trend for urban migration among tribal communities of India like other social groups [16]. In Tripura, such urbanization has led on the rise of a homogeneous sizable proportion of young adult tribal college students, who compared to non-tribal community students, have diverse food habits, ethno-linguistic and socio-cultural backgrounds.

Bone is a dynamic tissue that undergoes modeling and remodeling at different times and rates in response to a variety of stimuli throughout an individual's lifetime. Gains in peak bone mass are very rapid during adolescence, with at least 90% acquired by the age of 18 [1]. Longitudinal studies of changes in bone mass during growth have confirmed that in girls, the greatest increases in bone mass occur between the ages of 12–15 years, compared with 14–17 years in boys [2]. It is now well established that peak bone mass acquisition is largely determined by genetic and hormonal factors, but can be significantly influenced by life style factors, including body weight, dietary habits, smoking, sun exposure, and levels of physical activity [3]. Even though the clinical consequences of adverse bone health are largely seen in old age, evidence is accumulating that many predisposing factors to osteoporosis arise in childhood [4]. Several interconnected factors have been known to influence bone mass accumulation during growth. One of the most important modifiable factors in the development and maintenance of bone mass is nutrition [5] and undernourishment is one of the common features of osteoporosis. It has also been reported that poor nutrition is an important risk factor for development of osteoporosis in the elderly [6–9]. Assessments of nutritional status and individual nutrition correction additionally have been reported to reduce bone fragility and improve quality of life [6].

It is well-established that anthropometric device is an essential feature of nutritional evaluation for determining nutritional status of a particular community, like being overweight, obesity, muscular mass loss, fat mass gain, adipose tissue redistribution, skeletal health etc. Its indicators are used to evaluate the health status of a community and even for prognosis of chronic and acute diseases, and to guide medical intervention, if required, in people of all ages. Earlier several investigators all over the world used similar approach in investigating the anthropometric indices and nutritional status of the adults of different ethnic groups [17–24].

Biochemical markers of bone turnover have been shown to provide valuable information for the diagnosis and monitoring of metabolic bone diseases [25]. They reflect the whole body rates of bone resorption (Resorption markers) and bone formation (Formation markers). Therefore they may provide a more representative index of the overall skeletal bone loss

than would be obtained by measuring the rates of change in Bone Mineral Density (BMD) at specific skeletal sites [26].

The aim of this study was to examine the correlation between nutritional status with bone health of young adult college students of two different communities (Tribal and non-Tribal) by anthropometric measures and urinary indices.

2. MATERIAL AND METHODS

2.1 Subjects

This study was carried out during the period from October 2011 to March 2013. The studied population were from two diverse socio cultural origin, Kokborok ethno-linguistic tribal group (Group A) and Bengali ethno-linguistic non-tribal group (Group B), aged 18 to 21 years. The study area was selected in the semi-urban area to satisfy the prerequisite and similar environmental and climatic conditions of both the communities of subjects of this study with particular reference to exposure to sunlight. In Tripura, during the past one or two decades, urbanization among tribes has led on the rise of a homogeneous sizable proportion of young adult tribal college students, who compared to non-tribal community students, have had diverse food habits, ethno-linguistic and socio-cultural backgrounds with more receptiveness to western culture and food.

The area of this cross-sectional study in undergraduate colleges was intentionally selected because of higher distribution and concentration of the two groups of ethno-linguistically varied subjects in a common place, but with similar educational background. A multi-stage stratified random sampling method was utilized to finally select the subjects of this study. In the first stage, students of the two ethno-linguistic groups were identified from physical characteristics and surnames. The information provided by the subjects was subsequently verified from official records. In the next stage, random samplings was employed to select the subjects within the specific age group of this study and the subjects below or above the age (18-21 years) were excluded from study. The age of the subjects was further verified from official records and/or birth certificates. Next, all such randomly selected subjects were explained the objectivity and protocol of the research. In the subsequent stages, subjects were further screened based on their compliance or non-compliance for all kinds of tests and measurements, healthy or unhealthy, history of chronic disease or chronic medication or consumption of alcohol or tobacco use. Finally, only the voluntarily participated subjects with written consent were included in this study. The final sample size of both groups of subjects and their gender match however could not be achieved because of wide variation in ethnicity ratio (non-tribal 69: tribal 31) among the studied population. Thus, the studied population were from two diverse socio cultural backgrounds, tribal community (n=60; male: 30; female 30) and non-tribal community (n=100; male: 50; female: 50), aged 18 to 21 years. Ethical approval for human studies was obtained from the Advisory Committee of the Institutional Human Ethics Committee.

2.2 Anthropometric measurements

Each subject was measured for stature, weight, circumferences [mid upper arm circumference (MUAC), thigh circumference, fore arm circumference and calf circumference]

and skinfold thickness at desirable sites. All anthropometric measurements were made on the right side of the body by trained investigators by using the standard techniques [28-29].

Similar procedures were used to standardize height and weight measurements. Body weight was measured with a standard weighing scale to the nearest 0.1 kg with minimum clothing and standing height to the nearest 0.1 cm in the standard arm hanging position with Harpenden type Anthropometer. Triceps and subscapular skinfolds were measured to the nearest 0.1 mm with a Holtain skinfold caliper (Holtain Ltd.), and MUAC was measured with a metal tape, with the right arm hanging relaxed at the subject's side. MUAC was measured to the nearest 0.1 cm. Measurements were taken twice by the same trained person. The technical errors of measurement (TEM) were calculated by a standard formula:

$$TEM= \sqrt{\Sigma(\text{reading 1} - \text{reading 2})^2/2n}$$; where n is the number of subjects measured [30].

BMI was calculated as the weight in kilograms divided by the square of the height in meters. The nutritional status of individuals was evaluated according to internationally accepted World Health Organization (WHO) [31] guidelines for adults. CED III was defined as BMI less than 16.0, CED II as BMI of 16.0 to 16.9, CED I as BMI of 17.0 to 18.4, and normal as BMI of 18.5 to 24.9. We followed the WHO [31] classification of the public health problem of low BMI (<18.5), based on adult populations worldwide. According to this classification, a low prevalence (5%–9%) of low BMI is considered a warning sign requiring monitoring, a medium prevalence (10%–19%) as indicating a poor situation, a high prevalence (20%–39%) as indicating a serious situation, and a very high prevalence (≥ 40%) as indicating a critical situation.

For estimation of FFM, the percentage body fat was calculated by using Slaughter et al.'s skinfold thickness equations for adult males and for all females [32].

For estimation of MM, first corrected mid thigh girth (CMTG) and corrected calf girth (CCG) were calculated as [mid thigh girth – 3.14 X frontal thigh skin fold/10]^2 and [calf girth – 3.14 X mid calf skin fold /10]^2, respectively. Muscle mass (MM) was then estimated following the equation [33]:

$$MM= [\text{height X } \{(0.0553 \text{ X } CMTG^2)+(0.0987 \text{ X forearm gifth}^2)+(0.0331 \text{ X } CCG^2)\}-2445]/1000$$

Anthropometric prediction of SKM was performed by using the equation of Martin [34] as described elsewhere by Valtuena et al., [35]. Skeletal diameters of the elbow, wrist, knee and ankle were measured with Harpenden type spreading calipers to the nearest 1mm. Skeletal mass (SKM) was predicted using the equation of Martin [34]:

$$SKM\ (kg) = 0.60 \times 10^{-4} \times S \times (\sum b_i)$$

Where S is height in cm and bi are the individual skeletal diameters in cm.

2.3 Collection of urine samples and analysis

Daily urinary excretion of calcium, phosphate, creatinine, hydroxyproline were determined in 24hour urine sample. For this, the participants were given materials, oral and written guidance for home completion of 24 hour urine collection. They were instructed to consume modified diet free from meat for 1 week. Urine sample was collected on the 7th day after completion of this diet schedule. Subjects were further instructed to be free of any unusual

physical or mental stresses on the day of collection. Briefly, on the day of the collection, participants discarded their first urine void, recorded the time, and then collected all subsequent voids for 24 hour including a void at the recorded time the following morning. Samples reported to be incomplete were excluded. Urine samples were collected in polyethylene bottles containing 10 mL of 6N HCl as a preservative, sampled and stored at frozen temperature until the analysis was made. In female participants, urine was sampled from the 6th to the 12th day of the menstrual cycle to avoid changes in the composition of body fluids due to sexual hormones.

Urinary level of calcium was measured according to the method as described elsewhere by Kessler and Wolfman, [36] by using biochemical kits (LABKIT, CHEMELEX, S.A. Pol. Canovelles-Barcelona, Spain). Urinary phosphate, creatinine and hydroxyproline were measured according to the methods as described elsewhere respectively by Lowry and Lopez [37] Nath and Nath, [38] and Bergman and Loxley, [39] by using an analyzer (Microlab 300, E-Merck).

2.4 Quantitative ultrasound (QUS) measurement

Bone status was evaluated with quantitative ultrasound at the dominant calcaneus by the trained person by using the Achilles Express (GE Healthcare, Madison, WI, USA), a QUS device. In Achilles system, high frequency sound waves are used to evaluate bone status in the heel. It measures speed of sound (SOS) and broadband ultrasound attenuation (BUA) and combine them to form a clinical measure called the Stiffness Index (SI). T-scores were then generated against the Asian reference population database provided with the heel scanners. Before measurement, the instrument was calibrated daily in accordance with the manufacturer's recommendations. The manufacturer's cited precision error for the SI measurement is 2.4%. A T-score of > -1 was classified as normal, a score of < -0.1 and > -2.5 was classified as being at risk of having osteopenia while a T-score of < -2.5 was classified as at risk of having osteoporosis as per the classification of WHO [40].

2.5 Statistical analysis

All statistical tests were performed following standard techniques. Descriptive data were presented as mean ± SD. Pearson correlations and stepwise multiple regression analyses were performed. Unpaired t-tests were performed to check for differences in between the groups. Additionally, in QUS study, one-way analysis of variance (ANOVA) was performed to compare the group means. Pearson correlation coefficient (r) was used to study the relationship between bone turnover (skeletal health) markers (skeletal mass, calcium:creatinine ratio and hydroxyproline:creatinine ratio) and nutritional markers (BMI, MUAC, FFM and MM). In stepwise multiple regression analysis, skeletal mass (SKM) was used as dependent variable and corresponding independent variables were BMI, MUAC, FFM, calcium:creatinine (Ca:Cr) ratio, and hydroxyproline:creatinine (HPR:Cr) ratio. Statistical analyses were performed with SPSS, version 17.0. $P < 0.05$ was considered to indicate statistical significance.

3. RESULTS AND DISCUSSION

In the present communication, we report the results of a study with a select group of subjects where we examined the correlation between nutritional status and bone health of young college students of two different communities based on anthropometric and urinary indices, and quantitative ultrasound assessment. Data on a total of 160 adult college students were included in the analyses (Tribal – 60, Male: 30, Female: 30; Non-Tribal – Male: 50, Female: 50).

Table 1 depicts population-wise descriptive statistics (mean±standard deviation) of age, body weight and other anthropometric characteristics and derived indices between the two different communities of college students. Results indicate that, age as a variable, was not found significantly different among males and females of both communities. On the other hand, compared to females, males of both the communities were found to have significantly higher height (tribal $P < .001$, non-Tribal $P< .001$), body weight (tribal $P = .014$, non-Tribal $P< .001$), FFM (tribal $P< .001$, non-tribal $P< .001$), MM (tribal $P= .004$, non-tribal $P< .001$), SKM (tribe $P< .001$, non-tribal $P< .001$) and MUAC (non-Tribal $P< .001$). In case of tribal population, however, no such gender difference ($P= .290$) in MUAC was observed. A tribal vs. non-tribal comparison showed that, including age ($P= .038$), all the studied variables (height: $P < .017$, body weight: $P< .001$, MUAC: $P< .001$, FFM: $P< .003$, MM: $P< .001$, SKM: $P < .002$) were significantly different between the males of two communities, while, in case of females, except age ($P < .002$) and SKM ($P < .017$), no other variables were significantly different.

Table 1. Descriptive statistics and values of anthropometric characteristics of the young adult Tribal and non-Tribal college students

Variables	Tribe		Non – Tribe		*P*-value *			
	Male (I)	Female (II)	Male (III)	Female (IV)	I vs. II	III vs. IV	I vs. III	II vs. IV
Age (Years)	19.87 ± 0.86	20.07 ± 0.78	19.48 ± 0.65	19.48 ± 0.79	0.351	1.000	.038	0.002
Height (cm)	163.29 ± 4.20	152.91 ± 5.47	165.79 ± 4.79	151.63 ± 3.60	< 0.001	<0.001	0.017	0.258
Body Weight (kg)	53.61 ± 5.56	49.13 ± 7.84	59.30 ± 7.52	49.33 ± 5.78	0.014	<0.001	<0.001	0.908
BMI (kg/m^2)	20.12 ± 2.11	20.95 ± 2.70	21.58 ± 2.47	21.45 ± 2.46	0.190	0.788	0.006	0.404
MUAC (cm)	22.44 ± 1.34	22.91 ± 1.99	24.05 ± 1.89	22.66 ± 1.76	0.290	<0.001	<0.001	0.561
FFM (kg)	49.71 ± 4.17	42.02 ± 7.08	53.19 ± 5.84	40.91 ± 4.43	< 0.001	<0.001	0.003	0.441
MM (kg)	21.01 ± 3.44	17.84 ± 4.55	24.47 ± 4.36	17.95 ± 3.33	0.004	<0.001	<0.001	0.917
SKM (kg)	5.63 ± 0.61	4.47 ± 0.58	6.10 ± 0.66	4.17 ± 0.44	< 0.001	<0.001	0.002	0.017

BMI, body mass index; MUAC, mid-upper arm circumference; FFM, fat-free mass; MM, muscle mass, SKM, skeletal mass. All the values are expressed as mean ± SD.

** Significance level based on unpaired t-tests.*

Results of descriptive statistics and values of anthropometric characteristics among the two communities of students (Table 1) indicate that values for markers for nutritional status and bone health are significantly higher in males than females in both communities, except minor variations, and a tribal vs. non-tribal comparison also revealed community-based differences of values only among males. These suggest that among the participants of this study males of both communities are comparatively in a better condition as far as values for markers for nutritional status and bone health are concerned. Such variations in anthropometric characteristics between two different populations of diverse origin are consistent with those reported earlier by many workers [41–43].

Literature survey shows that, in several recent studies in India [44 – 49], BMI has been utilized to study the nutritional status of tribal populations. Earlier, several studies have well-documented the association and significance of CED with socio-economic, nutrition and health status of adult population [45, 50-53]. Therefore, this study was an effort to investigate the consequences of the functional impairments commonly associated with low BMI in subjects of two different communities having diverse food habits, ethno-linguistic and socio-cultural background.

Table 2 presents gender-wise nutritional status (BMI) of young adult college students of two different communities (tribal and non-tribal). The prevalence of CED, based on a BMI of less than 18.5 kg/m^2, was 10% (CED I) in non-tribal male, 12% (CED I) in non-tribal female, 30% in tribal male (CED I, 26.67%; CED II, 3.33%) and 20% in tribal female (CED I, 13.33%; CED II, 6.67%). When CED was assessed by BMI in overall population, 25% tribal students were affected, compared to 11% students of non-tribal community. As far as overweight and obesity of overall population are concerned, only 6.67% tribal and 8% of non-tribal background students were found overweight and there was no record of obesity among the total population studied.

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Table 2. Nutritional status of young adult Tribal and non-Tribal college students according to World Health Organization (WHO) [31] guidelines for adults BMI classification

Anthropometric Variables	Nutritional Status	Cut-off Value	Population				Population	
			Tribal		Non-Tribal		Tribal	Non-Tribal
			Male (n=30)	Female (n=30)	Male (n=50)	Female (n=50)	(n=60)	(n=100)
BMI	CED III	< 16.00	0%	0%	0%	0%	0%	0%
	CED II	16.00 – 16.99	3.33%	6.67%	0%	0%	5.00%	0%
(kg m^{-2})	CED I	17.00 – 18.49	26.67%	13.33%	10%	12%	20.00%	11%
	Total CED	***< 18.50***	***30 %***	***20%***	***10%***	***12%***	***25 %***	***11%***
	Normal	18.50 – 24.99	66.67%	70%	78%	84%	68.33%	81%
	Over weight I	25.00 – 29.99	3.33%	10%	12%	4%	6.67%	8%
	Obese	>=30.00	0%	0%	0%	0%	0%	0%

BMI, body mass index; CED, chronic energy deficiency

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The outcome of the present study clearly indicated that, when BMI was considered as a nutritional index, the highest prevalence of CED was noted in tribal males and lowest in non-tribal males (Table 2) suggesting that these two particular student groups of tribal and non-tribal background respectively were the maximum and minimum affected populations studied. However, an analysis with overall population indicated that prevalence of CED was higher in tribes (25%), compared to non-tribes (11%), suggesting that, although ethnic variations are there but students of both the ethnic backgrounds have nutritional insufficiency, which cannot be ignored and deserves immediate attention for corrective measures like nutritional intervention programs from local health authority through government, semi-government or private initiatives. The possible underlying mechanism for development of such nutritional insufficiency may be from socio-economic deprivation including lack of benefits from partial urbanization as both the population groups were selected from an identical socio-demographic background. Such recommendation for a nutritional and health surveillance finds support from WHO's [31] classification of the public health problem of low BMI (<18.5), based on adult populations worldwide. Similar report has been made earlier on tribal population who are at higher risk of undernutrition because of socio-cultural and socio-economic and environmental factors influencing the food intake and health seeking behavior [54]. Thus, anticipation of improvement in socio-economic conditions, better access to health services etc. in these semi-urbanized communities of students, irrespective of ethnic background, possibly was absent in the entire population studied. Support for such presumption comes from our observation of low prevalence of overweight and obesity in the total population studied, because prevalence of overweight and obesity has been linked with improvement of socio-economic conditions, urbanization, better nutrition, growing knowledge and awareness etc [55, 56].

Urinary excretion profile of markers of bone turnover in young adult college students of two different communities are summarized in Table 3. Except creatinine (P= .091) and phosphate (P= .515), significant gender-based differences in 24-hr excretion in calcium (P<0.001), hydroxyproline (P= .001), Ca:Cr ratio (P< .001) and HPR:Cr ratio (P< .001) were observed in students of tribal background. For students of non-tribal background, such significant gender-based differences in 24-hr excretion were observed for creatinine (P =0.042), hydroxyproline (P < .002), HPR:Cr ratio (P< .001) and phosphate (P< .001), while calcium (P= .072) and Ca:Cr ratio (P= .302) did not show any significant variation. A tribal vs. non-tribal comparison showed that, majority of the studied marker parameters, calcium (P= .001), hydroxyproline (P< .001), Ca:Cr ratio (P= .008) and HPR:Cr ratio (P< .001), were significantly different between the males of two communities, except creatinine (P= .052) and phosphate (P= .227), whereas, in case of females of two different communities, except hydroxyproline (P< .001) and HPR:Cr ratio (P< .001), no other markers were observed significantly different.

Table 3. Urinary excretion level of skeletal health marker parameters of young adult Tribal and non-Tribal college students

Variables	Tribal		Non – Tribal		*P*-Value*			
	Male (I) (n=30)	Female (II) (n=30)	Male (III) (n=50)	Female (IV) (n=50)	I vs. II	III vs. IV	I vs. III	II vs. IV
Creatinine (mmoL/24h)	9.28 ± 3.03	10.47 ± 2.25	10.82 ± 3.88	9.41 ± 2.93	0.091	0.042	0.052	0.072
Calcium (mmoL/24h)	12.86±2.00	9.79 ± 2.73	10.90 ± 3.32	9.62 ± 3.68	< 0.001	0.072	0.001	0.815
Hydroxyproline (mmoL/24h)	255.70 ± 117.01	174.28 ± 48.41	34.19 ± 15.68	46.21 ± 21.02	<0.001	0.002	<0.001	<0.001
Calcium : Creatinine	1.53 ± 0.54	1.03 ± 0.51	1.17 ± 0.63	1.06 ± 0.43	< 0.001	0.302	0.008	0.772
Hydroxyproline: Creatinine	29.19 ± 13.64	17.94 ± 8.32	3.28 ± 1.48	5.16 ± 2.52	<0.001	<0.001	<0.001	<.001
Phosphate	5.18 ± 2.27	5.56 ± 2.26	4.58 ± 1.83	6.41 ± 2.93	0 .515	<0.001	0.227	0.151

*All the values are expressed as mean ± SD. * Significance level based on unpaired t-tests.*

Nutrition is an important modifiable factor in the development of bone mass during adolescence [57] and the bone status of an individual or community includes not only the present bone size but also the direction in which likely to move [27]. Several studies also have demonstrated that 95-99% of peak bone mass is achieved by age 18 years (15-16 years in girls and 16-18 years in boys), which suggests that bone mass in late puberty may be prognostic factor for development of osteoporosis in the future [58, 59]. Also strong experimental or prospective evidence is not available regarding whether nutritional insufficiency impacts on bone health in younger population of late puberty of different ethnic backgrounds. As it has been suggested that nutrition is an important modifiable factor in the attainment of peak bone mass [57], which may be more relevant to future osteoporosis risk than bone loss in later life [60 - 62], the relationships between urinary bone marker indices and anthropometric nutritional indices may be particularly relevant in both communities. Thus, we assessed potential relationships between SKM, 24-h urinary calcium:creatinine, 24-h hydroxyproline:creatinine and anthropometric nutritional indices BMI, MUAC, FFM and MM.

Pearson's correlation coefficients between bone turnover markers (skeletal mass, Ca:Cr ratio, HPR:Cr ratio) and nutritional status markers (BMI,MUAC, FFM, MM) in students of tribal and nontribal backgrounds are summarized in Table 4. Correlation analyses indicated that, in case of non-tribal students, irrespective of gender, all the four independent variables were significantly positively correlated with skeletal mass (SKM). In case of tribal students, however, such significant positive correlation was not found for BMI in males and MUAC for females. Correlation analyses further indicated that, in case of non-tribal male, all the four independent variables were significantly inversely correlated with Ca:Cr ratio, whereas, in female, similar significant inverse correlation was seen only with BMI and FFM. In contrast, in tribes, all the four independent variables were significantly inversely correlated with Ca:Cr ratio in female, whereas, in male, similar significant inverse correlation was seen only with MM. Correlation coefficients of independent variables with hydroxyproline:creatinine ratio indicated that, in tribes, females were significantly inversely correlated with all variables, and in males such correlations were inverse but weak. However, BMI, in this case, showed weak positive correlation. In non-tribes, on the other hand, similar inverse significant correlation was seen only with FFM in male, and FFM and MM in female. Correlations with all other independent variables in both genders were inverse but weak.

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Table 4. Pearson's correlation coefficient of skeletal mass (SKM), calcium:creatinine ratio (Ca:Cr) and hydroxyproline:creatinine ratio (HPR:Cr) with body mass index (BMI), mid upper arm circumference (MUAC), fat free mass (FFM), muscle mass (MM) of young adult Tribal and non-Tribal college students

	Tribal (n= 30 Male, 30 Female)				Non-Tribal (n= 50 Male, 50 Female)			
	BMI	**MUAC**	**FFM**	**MM**	**BMI**	**MUAC**	**FFM**	**MM**
Skeletal Mass (SKM)								
Males	0.226	0.473**	0.686**	0.600**	0.377**	0.381**	0.604**	0.484**
Females	0.710**	0.319	0.901**	0.837**	0.528**	0.320*	0.571**	0.455**
Ca:Cr								
Males	-0.137	-0.061	-0.321	-0.398*	-0.320*	-0.325*	-0.356*	-0.336*
Females	-0.425*	-0.402*	-0.559**	-0.459**	-0.293*	-0.194	-0.313*	-0.231
HPR:Cr								
Males	0.059	-0.142	-0.333	-0.183	-0.196	-0.248	-0.301*	-0.206
Females	-0.412*	-0.488**	-0.421*	-0.446*	-0.262	-0.185	-0.345*	-0.300*

* denotes significance level $P<0.05$ and ** denotes $P<0.01$

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We found significant positive correlations between skeletal mass and anthropometric nutritional indices, while significant negative correlations between calcium: creatinine, hydroxyproline: creatinine ratios and anthropometric nutritional indices, with few community and gender variations. In our participants, skeletal mass, a bone health marker, and nutritional indices were correlated positively and strongest correlations were found among tribal females, particularly with FFM and MM, followed by tribal males and non-tribal population. Conversely, Ca:Cr ratio and HPR:Cr ratio, two bone resorption markers, and anthropometric nutritional indices were correlated negatively and strongest correlations were found among tribal females, particularly with FFM, MM and MUAC, followed by non-tribal population and tribal males. These results thus provide suggestive evidence that nutritional status as predicted by anthropometric indices possibly had a modifying role over bone health in our participants. Supportive data for similar conclusion were obtained earlier by Vatanparast et al., [57]. As far as anthropometric nutritional indices as potential predictor of skeletal health is concerned, FFM in our participants was found strongly associated with skeletal mass explaining 17% to 81% variance, suggesting that in anthropometry-based population study, FFM may be recommended as a simple anthropometric estimate to assess nutrition and skeletal health status of any adult population.

Stepwise multiple regression analysis between skeletal mass (dependent variable) and BMI, MUAC, FFM, Ca:Cr and HPR:Cr ratio (independent variables) are summarized in Table 5. Results indicated that, when BMI, MUAC, FFM, MM, Ca:Cr ratio and HPR:Cr ratio were considered as potential predictors, FFM proved to be the predominant predictor for skeletal mass, irrespective of gender and community background, with values for R^2 change ranging from 17% to 81%.

Table 5. Stepwise multiple regression analysis of all the subjects between SKM (dependent variable) and BMI, MUAC, FFM, MM, Ca:Cr, HPR:Cr (independent variables)

	SKM			
Tribal (Male)	**R^2 change**	**β**	**Standard β**	**P - value**
BMI				> 0.05
MUAC				> 0.05
FFM	0.470	0.069	0.470	< 0.001
MM				> 0.05
Ca : Cr	0.067	- 0.325	- 0.287	0.018
HPR : Cr	0.188	- 0.017	- 0.372	0.003
Tribal (Female)				
BMI				> 0.05
MUAC				> 0.05
FFM	0.811	0.074	0.901	< 0.001
MM				> 0.05
Ca : Cr				> 0.05
HPR : Cr				> 0.05
Non-tribal (Male)				
BMI				> 0.05
MUAC				> 0.05
FFM	0.364	0.044	0.387	< 0.001
MM				> 0.05
Ca : Cr	0.073	- 0.319	- 0.304	0.004
HPR : Cr	0.189	- 0.161	- 0.361	0.001
Non-tribal (Female)				
BMI				> 0.05
MUAC				> 0.05
FFM	0.170	0.044	0.435	< 0.001
MM				> 0.05
Ca : Cr	0.326	- 0.454	- 0.435	< 0.001
HPR : Cr				> 0.05

SKM, skeletal mass; BMI, body mass index; MUAC, mid-upper arm circumference; FFM, fat-free mass; MM, muscle mass, Ca:Cr, calcium;creatinine ratio; HPR:Cr, hydroxyproline;creatinine ratio.

Results of QUS measurement are summarized in table 6. Results showed that stiffness index differ significantly among the groups (F=4.180, *P* < .01). Males of non-tribal population showed the highest stiffness index followed by tribal male, non-tribal female and tribal female. However, T-scores among these groups did not differ significantly (F=2.454, *P*= .067). When we applied the specific T-score designations, based on the World Health Organization (WHO) criteria [40], to the calcaneal QUS values, 80%,77%, 56.67% and 23.33% populations respectively from non-tribal male, tribal male, tribal female and non-tribal female groups were found with normal BMD (T scores of >-0.1). Non-tribal (76.67%) and tribal (33.33%) females were found under severe threat of osteopenia (T-scores of < −0.1 and > −2.5). Additionally, 10% of the tribal females were found having osteoporotic changes (T-score of < −2.5).

Table 6. Quantitative ultrasound measurement (stiffness index and T-score) of the calcaneus in young adult Tribal and non-Tribal college students.

Population	Stiffness Index	T-Score	Normal BMD	Osteopenia	Osteoporosis
Tribal Male	94.17 ± 11.71	– 0.45 ± 0.90	77.00%	23.00%	
Tribal Female	87.53 ± 15.20	– 0.76 ± 1.16	56.67%	33.33%	10.00%
Non-tribal Male	98.20 ± 14.80	– 0.11 ± 1.13	80.00%	20.00%	
Non-tribal Female	88.20 ± 12.43	– 0.70 ± 0.97	23.33%	76.67%	

Values are expressed as mean ± SD. Significance levels among the stiffness indices and T-scores were P<0.01 and P>0.05 respectively based on one way ANOVA.

Quantitative ultrasound (QUS) has been shown to be a valid technique in the non-destructive evaluation of the elastic properties of bone tissue in vitro [63]. QUS is particularly attractive because it is simple, inexpensive, portable, non-invasive and free of ionizing radiation. As such QUS has much greater potential for widespread application than traditional X-ray bone densitometry approaches [64]. Data generated from QUS studies revealed that irrespective of gender and community there was a disturbing prevalence of osteopenia and even osteoporosis in our studied population who had just completed pubertal growth. This together with our results of nutritional scores (CED based on BMI) (Table 2) and its strong relationship with skeletal mass (Table 4) give empirical support to provide suggestive evidence that nutritional insufficiency may have adverse effects on bone.

As far as limitations of this study are concerned, it may be its small and unequal sample size, particularly for tribal group and use of QUS in assessing bone health. But investigators had no alternative in these issues because (i) the total tribal population of the state is only 31%, (ii) only a smaller fraction of this population usually enrolls for college level education, (iii) many subjects of this group were either discarded or dropped during the multi-stage stratified sampling method and (iv) the lack of technical facilities of dual-energy X-ray absorptiometry (DEXA) which led us to use alternative technique QUS for assessment of bone mineral density (BMD).

4. CONCLUSION

In conclusion, observations of this study provide suggestive evidence that nutritional insufficiency may cause adverse effect on bone.

CONSENT

All authors declare that written informed consent was obtained from all the participants.

ETHICAL APPROVAL

Ethical approval for human studies was obtained from the Advisory Committee of the Institutional Human Ethics Committee.

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ABBREVIATIONS

BMI,	body mass index
MUAC,	mid-upper arm circumference
FFM,	fat-free mass
MM,	muscle mass
SKM,	skeletal mass
CED,	chronic energy deficiency
Ca:Cr,	calcium : Creatinine ratio
HPR:Cr,	hydroxyproline: Creatinine ratio
SKM,	skeletal mass
QUS,	quantitative ultrasound