EFFECTS OF THYROIDAL DYSFUNCTIONS ON BONE MARKERS IN NON-PREGNANT WOMEN: A HOSPITAL BASED CROSS SECTIONAL STUDY FROM KHYBER PAKHTUNKHWA NORTHERN PAKISTAN

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ABSTRACT

Objective: To study the ill effects of thyroid dysfunctions on biochemical markers of bone formation in non-pregnant women (NPW) of Khyber Pakhtunkhaw in Northern Pakistan.

Methodology: This cross sectional study was conducted in Khyber Teaching Hospital. The study group comprised of 258 non-pregnant women in the age range of 18-75 years. Thyroid profile was determined by Elisa methods for all the patients. Serum level of alkaline phosphatase (ALP), calcium and zinc were also determined using standard protocols and the data was analyzed statistically, using SPSS version 21.

Results: Mean serum calcium was highest in overtly hyperthyroid (OH) group (9.80 ±0.90mg/dl) and lowest in overtly hypothyroid (Oh) group (8.95± 0.10 mg/ dl). Serum total alkaline phosphatase was found to be maximum in normal (N) group (159± 7.61 U/l) and minimum in sub clinically hypothyroid (Sh) group (128± 6.42 U/l). There were no significant differences in the serum zinc (Zn) level of all the diseased groups. Calcium showed significant positive correlation with TSH in Oh (p=0.01) and OH (p=0.04). Serum calcium was significantly negatively correlated with T_3 in OH (p=0.01). Serum alkaline phosphatase was significantly negatively related with T_4 in OH (p=0.01). Serum Zn showed a very significant positive correlation with T_3 in sub-clinical hyperthyroid (SH) (p=0.02).

Conclusions: Thyroid dysfunctions may disturb bone formation and resorption equilibrium.

Key Words: Thyroid dysfunction, Bone markers, Calcium, Alkaline phosphatase

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INTRODUCTION

Thyroid hormones are believed to disturb bone formation and resorption equilibrium, which are necessary for the normal architecture of bone. Thyroid diseases are considered to be one of the major causes of secondary osteoporosis in human beings[1]. This effect is mediated through different biochemical mechanisms. Many studies have revealed that thyrotoxicosis causes bone diseases[2]. Data show that hypothyroidism delay the bone remodeling process and also reduces bone turnover. Large population based studies have shown that hypothyroidism increases the fracture risk two- to three-fold[3].

Thyroidal hormones are also necessary for bone development and maintaining it's optimal strength. Population based studies show that thyroidal dysfunctions are linked with high fracture risk[4].

Biochemical bone biomarkers shows significant changes in bone minerals turnover in thyroidal dysfunctions. Hypothyroidism decreases rate of bone formation and also destroy bone architecture. Hypothyroidism for more than one year will decrease bone age[5]. Bone density is higher than normal in adult hypothyroid patients. It is generally linked with low serum ALP, normal level of serum calcium and phosphorus[6]. Hypothyroid subjects may develop hypercalcaemia due to intolerance to oral calcium. This defect is due to the decrease in the rate of calcium deposition, rather than its increased absorption. In hyperthyroidism, patients have high levels of serum calcium and are associated with low bone mineral density (BMD)[7].

Serum Alkaline Phosphatase (ALP) level is found to

be abnormal in thyroid dysfunctions. Bone specific ALP is a better biomarker than total alkaline phosphatase[8]. Its level is usually increased in hyperthyroidism[9]. It has been reported that bone loss due to thyrotoxicosis does not depends upon the concentration of circulating TSH levels. Thyroid hormones are believed to influence calcium metabolism. Hyperthyroidism elevate while hypothyroidism lower serum calcium level[10].

Thyroidal dysfunctions are more prevalent in females and its adverse effects in pregnant females are well established[11,12]. According to a WHO report on the nutritional value of vitamins and minerals, the prevalence of goiter in pregnant women (PW) is 22.2% while in non-pregnant women (NPW) is 20.9 % respectively. 2.5 billion population is suffering globally from iodine deficiency, out of which 313 million live in the South-Eastern Asian countries including Pakistan[13].

The northern areas of Pakistan are considered to be iodine deficient. The goiter prevalence rate is found to be 55% in plains to 80-90% in the northern mountainous region[14,15]. The prevalence rate of hyperthyroidism in Northern Pakistan is 5.1% with higher prevalence in females than in males[16]. The data about ill effects of thyroid dysfunctions on bone formation markers in non-pregnant women (NPW) is limited especially in south Asia in general and Pakistan in particular. The aim of the present work was to study the effects of thyroid dysfunctions on bone formation markers in non-pregnant women (NPW) of Khyber Pakhtunkhwa.

METHODOLOGY

This hospital based cross sectional study was conducted from March 01, 2014 to March 31, 2015 in Khyber Teaching Hospital (KTH), Peshawar, a tertiary care hospital in Khyber Pakhtunkhwa (KP) province of Northern Pakistan. The study was approved by the ethical committee of the Khyber teaching Hospital (KTH), through its letter no 21876/KTH/ P.S. The study population was selected from the patients visiting various outpatient departments of KTH for thyroid screening using purposive sampling method. The exclusion criteria were diseases like kidney, liver, bone, hypertension and diabetes.

The study group fulfilling the purpose of the study was 204 non-pregnant women in the age group of 18-75 years. The study group was sub-divided into four groups based on their thyroid profile status as Oh, Sh, OH and SH respectively. Control group (N) comprising of 54 non-pregnant women were recruited from the general population of Peshawar valley.

Informed consent was sought from each patient and all human dignity was respected throughout the study period in accordance with the international norms involving human as an experimental subjects. 5 ml of fresh venous blood (fasting sample) was taken from each patient. It was divided into two portions- one portion was used for the determination of thyroid profile markers by Elisa methods and the other portion was used for the determination of serum alkaline phosphatase, serum calcium and serum Zn level using standard protocols.

Thyroid profile markers (Serum TSH, T_4 and T_3) were determined using ELISA kits obtained from Biocheck, Inc. catalog Number: BC-1001, BC-1007 and BC-1005 respectively on Dia 710 micro plate reader (Made in Australia). Serum Triiodothyronine (T_3) and Tetraiodothyronine (T4) were determined using Competitive ELISA methods[17,18] and TSH was analyzed by Sandwich ELISA method[19]. The normal values for TSH, T_4 and T_3 were 0.4- 6.0 µ IU/ml, 4.8-12.0 µg/dl, and 0.6-1.85 ng/ml respectively.

Alkaline Phosphatase was determined on autoanalyser (Erbamannhein chemistry autoanalyser, Germany) using standard Erba kits according to IFCC recommendation utilizing 4-nitrophenyl phosphate as the substrate[20]. The normal range was 80-360 U/l. Serum calcium was determined by direct colorimetric method involving the complexation of serum calcium with Arsenazo III, to form a coloured chromophore, the absorbance of which was measured at 650 nm using autoanalyser (Erbamannhein chemistry autoanalyser, Germany) using standard Erba kits[21]. The absorbance was proportional to calcium concentration in the serum. The normal range was 8.6-10.2 mg/dl. Serum Zn was determined on atomic absorption (Perkin Elmer AAS 700) using flame technique on graphite furnace[22].

The data was statistically analyzed using SPSS for windows 21.0 software and Microsoft Excel. Values were reported as mean ± standard deviation. Pearson's correlation of the data was also carried out to look for association between variables. A p value of <0.05 was considered to be significant.

RESULTS

The study group and the control group (258 non-pregnant women) were age matched. The mean age of patients in the control group (N) was 42.15 ±1.86 years, 46 ±1.38 years for overtly hypothyroid (Oh), 45.97 ±1.93 years for sub-clinically hypothyroid(Sh), 49.74 ±1.62 years for overtly hyperthyroid (OH) and 48.94 ±1.87 years for the sub-clinically hyperthyroid (SH). The control group were 54, of which 61.11%(33) were menopausal (M, age below 45 years), 18.52%(10) were early post-menopausal (EPM, age 45-50 years) and 20.37%(11) were late post-menopausal (LPM, age above 50 years). The %age of M, EPM and LPM in the Oh(48) and Sh(48) group were, 54.16%(26), 12.5%(06), 33.83% (16) and 45.83% (22), 22.92% (11), 31.25% (15) respectively. Similarly the %age of M, EPM and LPM in

the OH (58) and SH (50) were 34.48% (20), 27.58% (16), 37.93% (22) and 43.14% (22), 07.84% (04), 49.07% (25) respectively.

The values of thyroid hormones and biomarkers of bone formation are presented in table 1. The mean serum level of TSH was found to be lowest in OH group (0.17± 0.01 µIU/ml) and highest in the Oh group (25.89± 2.86 µIU/ml). Serum T_3 level was highest in OH group (2.37± 0.01 ng/ml) and lowest in Oh group (0.94± 0.09 ng/ml). Highest serum T_4 level was found for OH group (12.07± 0.46 µg/ dl) and lowest in Oh group (4.30± 0.28 µg/dl). Mean serum calcium was highest in OH group (9.80± 0.90 mg/dl) and lowest in Oh group (8.95± 0.10mg/dl). Serum ALP was found to be maximum in N group (159± 7.61 U/l) and minimum in Sh group (128± 6.42 U/l). No significant differences were found in the serum zinc level of all the diseased groups.

Regression analysis of thyroid profile with bone markers in the study groups are shown in table 2 to 6. Serum calcium was negatively related with TSH in N, OH and SH while positively in Oh and Sh groups. Serum calcium was positively related with T_3 in all groups except OH group where it was negatively related. With T_4 it was negatively related in Sh and SH groups and positively related in the rest of the groups.

Serum alkaline phosphatase was negatively related with TSH in Sh and SH and positively related in the other groups. It was negatively related with T_3 except in Sh group. With T_4 it was positively related in Oh and SH and negative in the rest of the groups. Serum zinc was positive with TSH in all the groups. With T_3 it was negatively related in N and Sh and positively related in the rest of the groups. It was negatively related withT_4 in SH and positively related in the other groups.

Correlation analysis of thyroid profile markers and bone formation markers are presented in table 7 to 9. Serum calcium showed significant positive correlation with TSH in Oh (p=0.01) and OH (p=0.04). Serum calcium was significantly negatively correlated with T_3 in OH (p=0.01). No significant correlation was found between serum calcium and T_4 in any group.

Serum alkaline phosphatase was significantly negatively related with T_4 in OH (p=0.01). No significant correlation was found for it in the other study groups. Serum Zn showed a very significant positive correlation with T_3 in SH (p=0.02). No significant correlation was found for it with other thyroid profile markers in the other study groups.

Table 1: Thyroid profile and bone markers in non-pregnant women

S. No.	Group ID	Frequency(n)	Thyroid profile markers			Bone turn over Markers		
			TSH	T3	T4	S. Ca	ALP	S. Zn
1	N	54	2.38± 0.49	1.68±0.05	8.50±1.59	9.20±0.75	159±7.61	0.45±0.11
2	Oh	48	25.89±2.86	0.94±0.09	4.30±0.28	8.95±0.10	138±5.30	0.56±0.08
3	Sh	48	19.04±2.22	1.47±0.08	7.07±0.38	8.96±0.08	128±6.42	0.60±0.10
4	OH	58	0.17±0.01	2.37±0.01	12.07±0.46	9.80±0.90	144±7.12	0.61±0.11
5	SH	50	0.18±0.01	1.64±0.04	8.16±0.24	9.58±0.10	142±4.97	0.61±0.11

N: Normal, **OH**: Overtly hyperthyroid, **SH**: Sub clinically hyperthyroid, **Oh**: Overtly hypothyroid, **Sh**: sub clinically hypothyroid.

TSH: Thyroid Stimulating Hormone, **T3**: Triiodothyronine, **T4**: Tetraiodothyronine

Table 2: Regression analysis of thyroid profile with bone markers in the normal group (N)

Model		Unstandardized Coefficients					
DV		S. Ca		ALP		S. Zn	
Constant		B	SE	B	SE	B	SE
		8.64	0.74	236.04	53.91	0.32	0.11
IV	TSH	-0.06	0.08	3.53	5.94	0.01	0.01
	T_3	0.02	0.30	-29.91	21.56	-0.02	0.04
	T_4	0.08	0.07	-3.86	4.86	0.02	0.01

IV: Independent Variable**, DV:** Dependent Variable, **SE:** Standard Error

Table 3: Regression analysis of thyroid profile with bone markers in the Overtly hypothyroid group (Oh)

Model		Unstandardized coefficients					
DV		S. Ca		ALP		S. Zn	
Constant		B	SE	B	SE	B	SE
		8.16	0.34	132.91	18.55	0.38	0.06
IV	TSH	0.02	0.01	0.01	0.29	0.001	0.001
	T_3	0.25	0.16	-10.68	8.71	0.04	0.03
	T_4	0.04	0.06	3.01	3.05	0.00	0.001

Table 4: Regression analysis of thyroid profile with bone markers in the sub clinically hypothyroid group (Sh)

Model		Unstandardized coefficients					
DV		S. Ca		ALP		S. Zn	
Constant		B	SE	B	SE	B	SE
		8.45	0.37	133.32	33.14	0.51	0.08
IV	TSH	0.01	0.01	-0.29	0.54	0.00	0.001
	T_3	0.39	0.16	2.42	13.92	-0.004	0.03
	T_4	-0.04	0.03	-0.46	2.95	-0.01	0.01

Table 5: Regression analysis of thyroid profile with bone markers in the Overtly hyper thyroid group (OH)

Model		Unstandardized coefficients					
DV		S. Ca		ALP		S. Zn	
Constant		B	SE	B	SE	B	SE
		10.16	0.58	198.33	34.86	0.49	0.08
IV	TSH	2.08	1.26	81.48	76.60	0.19	0.17
	T_3	-0.38	0.18	-7.39	10.73	0.03	0.02
	T_4	0.02	0.04	-4.16	2.15	0.001	0.01

Table 6: Regression analysis of thyroid profile with bone markers in the sub clinically hyperthyroid group (SH)

Model		Unstandardized coefficients					
DV		S. Ca		ALP		S. Zn	
Constant		B	SE	B	SE	B	SE
		10.23	0.60	134.12	29.71	0.45	0.09
IV	TSH	-1.26	1.10	-14.68	54.74	0.11	0.17
	T_3	0.39	0.36	-19.60	17.93	0.12	0.06
	T_4	-0.13	0.07	5.29	3.29	-0.01	0.01

Table 7: Correlation analysis of bone markers with TSH in the various groups

Parameter	Group ID	Bone markers					
		S. Ca		ALP		S. Zn	
TSH		r	p	r	p	r	p
	N	-0.13	0.36	0.15	0.28	0.09	0.51
	Oh	0.34*	0.01	0.02	0.88	-0.17	0.24
	Sh	0.133	0.39	-0.01	0.50	-0.02	0.92
	OH	0.269*	0.04	0.22	0.11	0.10	0.46
	SH	-0.21	0.11	-0.11	0.40	0.14	0.33

*p <0.05 level (2-tailed significant)

Table 8: Correlation analysis of bone markers with T3 in the various groups

Parameter	Group ID	Bone markers					
		S. Ca		ALP		S. Zn	
T_3		r	p	r	p	r	p
	N	0.07	0.60	-0.24	0.07	-0.40	0.80
	Oh	0.19	0.21	-0.15	0.30	0.08	0.58
	Sh	0.21	0.15	0.07	0.64	-0.21	0.15
	OH	-0.33*	0.01	-0.24	0.07	0.18	0.18
	SH	-0.01	0.96	0.03	0.80	0.32*	0.02

*p <0.05 level (2-tailed significant).

Table 9: Correlation analysis of bone markers with T4 in the various groups

Parameter	Group ID	Bone markers					
		S. Ca		ALP		S. Zn	
T_4		r	p	r	p	r	p
	N	0.17	0.22	-0.15	0.27	0.21	0.13
	Oh	0.01	0.97	0.01	0.53	0.11	0.47
	Sh	-0.12	0.41	0.03	0.84	0.14	0.34
	OH	-0.11	0.43	-0.34**	0.01	0.08	0.53
	SH	-0.15	0.25	0.19	0.16	0.08	0.56

*p <0.05 level (2-tailed significant).

DISCUSSION

Thyroid dysfunctions often affect bone turn overs. In most population based studies, it has been found that both over activity (hyperthyroidism) and under activity (hypothyroidism) of thyroid glands are associated with high fractures risk. In the present study we attempted to assess the changes in bone formation biomarkers in various thyroid functional disorders. The markers included in our study were very simple and can be measured easily in any clinical laboratory except Zn, which is measured by atomic absorption.

In the present study mean serum calcium was found to be highest in OH group (9.80± 0.90 mg/dl) and lowest in Oh group (8.95± 0.10 mg/dl as compared to N group (9.20± 0.75 mg/dl). These results of our study are in agreements to other similar studies[5,6]. In hyperthyroidism, resorption of bone releases calcium in the blood and elevate its level while in hypothyroidism overall metabolism slows down which leads to low absorption of calcium from the gut and more excretion in urine which lower calcium level[21,23].

Thyroid hormones increase the osteoblastic activity which leads to demineralization of bone than its mineralization. The exact mechanism of this imbalance is unknown. According to one view, deficiency of T_3 decreases the activity of osteoclast and hence calcium level is reduced, while in hyperthyroidism the opposite effects are observed.

In our study the serum ALP level was higher in hyperthyroid than hypothyroid patients. This change in ALP

level was not due to liver dysfunctions because their ALT, AST were within the normal range as we observed in our other part of this study. This abnormal level was most probably associated with thyroid disorders and not liver dysfunction. It is more likely due to altered bone metabolism. Bone specific alkaline phosphatase is considered to be a superior marker than total alkaline phosphatase because it originates only from osteoblast in bone[6,13]. Its activity is elevated in hyperthyroidism and is reduced in hypothyroidism[24]. Serum levels of bone specific ALP show osteoblastic activity. High levels in hyperthyroidism indicate high osteoblastic activity and thus increased bone resorption than its formation. These observations of our study are in good agreement with the other similar studies[9].

According to our view it was the first kind of study from this part of Pakistan although similar studies are reported from other parts of the world. In northern Pakistan there is a lack of health care facilities, especially in rural areas. Moreover the gender differences and lack of health awareness in women folk as another major hurdle in the early diagnosis of thyroidal dysfunction in females. The findings of our study will encourage other researchers and health care provider from this area to redress the gravity of the problems to reduce the social and financial implications of the problem.

LIMITATIONS

The study has got a number of limitations which could not be minimized despite our best efforts. The most important limitation was the socio-cultural barriers, hindering the collection of data from the patients, regarding family medical history, medications and economic conditions. Other bone formation markers like bone specific alkaline phosphatase which are considered better marker were not measured due to lack of laboratory facilities and financial constraints. Lastly the study was carried out in one centre, involving only 258 non pregnant women and hence its findings cannot be generalized. Further studies are required involving larger populations for better results.

CONCLUSION

Bones resorption occurs at higher rate in hyperthyroid patients and bone turnover is increased in favor of resorption. Opposite changes were observed in hypothyroid women.

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CONTRIBUTORS

JS Conceived the idea and final revision of the manuscript. MSJ helped Study design. MY did literature survey and manuscript writing All authors contributed significantly to the submitted manuscript.