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Asymptomatic Osteomalacia in Pregnancy

ABSTRACT

Background:
Alkaline phosphatase (ALP) is important enzyme in diagnosis many diseases like osteomalacia. It presents in defect isoenzyme including bone, liver, and placental ALP.

Aims of this study:
Assess the efficacy of the bone ALP enzyme and intact parathyroid hormone (iPTH) to detect the possible presence of osteomalacia in pregnant women due to the vitamin D deficiency.

Objective:
Evaluate the nutritional state of pregnant women that will affect the good health of her bone and preparing her for lactation stage.

Methods:
Blood samples were collected from 87 normal fasting pregnant women then subdivided them into three subgroups according to their period of gestation. ALP and its isoenzyme were determined calorimetrically after separation of them by thermal stability method, and the level of parathyroid hormone was measured for 36 of them from different subgroups by the ELISA technique. Statistical test used as t-test (student test) and leaner regression .

Results:
There was a difference in the activity rates of the heat labile enzyme (HLAP), which is mostly related to the bones when compared between groups, and showed that there is a positive relationship between the activity of this enzyme with the activity of the placental isoenzyme and with the gestational age. PTH showed an increasing in its level and a direct proportion with placental isoenzyme.

Conclusion and recommendation:
Increased bone (ALP) isoenzyme, and elevated PTH could be a marker of osteomalacia in pregnancy, accordingly, we recommend a measurement of parathyroid hormone and placental, bone ALP in pregnant women to ensure the health of the placenta and bone.

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Introduction:

ALP activity usually increases during pregnancy; this is mostly due to increased placental isoenzyme production and bone isoenzyme production. (1). Because this contributions of the placental fraction, it is not a useful marker of bone formation in pregnancy (2) so alkaline phosphatase isoenzyme analysis is used to understand the changes in serum values of ALP and has many diagnostic advantages in identifying underlying pathology. The methods that used for isolation of isoenzyme includes : thermo stability test , inhibition with various small peptides and immunologic methods (3). Heat stable alkaline phosphatase (HSAP) represents the placental isoenzyme of alkaline phosphatase which is determined by the heat stability test that is done by heating at 65°C for 30 minute, other isoenzyme will be suppressed so called heat labile ALP (HLAP), this provides the most convenient and specific test for this isoenzyme. (4). HLAP includes (mostly liver and bone but less intestinal ALP).

Hepatic disorders in pregnancy are rare, (5), and liver isoenzyme elevated only with liver diseases, on other hand, most of activity of intestinal isoenzyme can be ignored through sampling a fasting state (6). The maternal bone formation may be increased during pregnancy (7) and presumably this increase is compensatory, allowing for replacement of bone lost to resorption when calcium is mobilized in order to meet the fetal demand because of calcium and phosphorous transfer from mother to fetus through placenta via an active mechanism (8, 9), so placental isoenzyme facilitates the mobilization of calcium from the maternal system for the fetal calcification process. Also it is responsible for active transport of phosphate.

Normally Serum iPTH levels are significantly lower during pregnancy as compared to no pregnant state (10).

Parathyroid hormone regulates feto-placental mineral homeostasis and skeletal development and stimulates

placental calcium transfer (11). The regulation of PTH secretion involves several mediators, including extra cellular calcium, vitamin D and magnesium (12, 13). Placental calcium transfer is stimulated by parathyroid hormone, which controls feto-placental mineral homeostasis and skeletal growth (11). PTH secretion is regulated by a number of mediators, including calcium, and vitamin D (12, 13). during pregnancy, there is an inverse association between vitamin D level and PTH (14), and there is high deficiency in vitamin D (15), this happen because deficient vitamin D lead to reduced calcium absorption through intestine leading to increase PTH to compensate hypocalcaemia on expense of bone content.

MATERIALS and METHODS

This study represents a cross-sectional study, eighty seven fast healthy pregnant female, their ages ranged from 14-42 years with mean of (24.78) were included and they divided into three subgroups according to their gestational pregnancy age. Blood samples were collected The placental isoenzyme of alkaline phosphatase was determined after separation by using the heat stability test, which is done by heating the serum at 65°C for 30 minute, using "Helena Laborites oven" then colorimetric method used to measure ALP and heat stable ALP (HSAP) activities (4)

Heat Labile ALP (HLAP) = Total ALP activity - Heat stable ALP.

HLAP represent bone, liver, and intestinal ALP while HSAP represent placental ALP. measurement of Intact PTH concentration is made by ELISA technique .The statistical methods were used for the analysis were standard statistical methods to determine the mean (x), Unpaired (student's) t-test to compare between trimesters with each other's, and Linear regression (r) for finding the degree of association between the parameters, Logarithmic transformation done to manage the data which are not normally distributed (16), then anti logarithmic value calculated

all values were considered not significant at p> 0.05 statistical analysis was conducted by using SPSS.

RESULTS

In this study we find that:

1- The mean of ALP in the 87 pregnant women was (9.07 K.A.U.\100ml) and its range was (3.51-25.80), mean of HLAP was (5.9 K.A.U.\100ml), and its range was (2.1 – 15.8 K.A.U.\100ml). Mean of HSAP was (3.3 K.A.U.\100ml) and its range was (1– 16.25 K.A.U.\100ml) as in table-1.

Table -1: Mean and range of ALP, HLAP, HSAP

Parameter	*Pregnant (n)*	*Mean (Range)*
ALP (K.A.U./100ml)	*87*	*9.07 (3.51-*
HLAP	*87*	*5.9 (2.1 – 15.8)*
HLAP 1ST trimester	*17*	*3.76*
HLAP 2nd trimester	*43*	*5.97(2.1 – 13.6)*
HLAP 3rd trimester	*27*	*7.11*
HSAP	*87*	*3.3 (0.65 – 16.25)*
HSAP 1ST trimester	*17*	*1.69 (0.8 – 3.85)*
HSAP 2nd trimester	*43*	*2.91 (0.65 – 13.6*
HSAP 3rd trimester	*27*	*4.92 (1 - 16.25)*

2- The mean of intact PTH level in the pregnant women was (80.52 Pg. /ml) and its range was (6 – 270 Pg. /ml), and the mean of Anti. PTH logarithm (46.77 Pg. /ml) which its range was (6.03 – 269.15 Pg. /ml) as in table -2.

Table -2: Mean and range of PTH, and Anti-PTH.log

PTH (Pg./mL)	*36*	*80.52 (6 – 270)*
Anti PTH Log (Pg /mL)	*36*	*46.77 (6.03 – 269.15)*

3- Table-3 appear that comparison of the means of HLAP in each trimester of Pregnancy (3.76, 5.97, and 7.11) respectively a highly significant ($p<0.001$) difference between 1st and 2nd, 3rd trimesters appear.

The difference between 2nd and 3rd trimesters was non-significant ($p>0.05$) as in table -3, also there is a significant ($p<0.004$) difference in serum HSAP between means of 1st trimester (1.69) and 2nd trimester (2.91), and significant difference ($p<0.005$) between means of 2nd trimester (2.91) and 3rd trimester (4.92) of pregnancy and highly significant difference ($p<0.001$) between 1st and 3rd trimesters in table -3.

Table -3: Independent sample t –test show the differences between the mean of HLAP, HSAP in each trimester

Parameter	Trimester	Mean	p
HLAP K.A.U./100 ml	1st 2nd	3.76 5.97	< 0.001
	2nd 3rd	5.97 7.11	0.24 NS
	1st 3rd	3.76 7.11	< 0.001
HSAP K.A.U./100 ml	1st 2nd	1.69 2.91	< 0.004
	2nd 3rd	2.91 4.92	< 0.005

	1st 3rd	1.69 4.92	< 0.001

There was highly significant (p<0.001) relationship between HLAP and the gestational age during pregnancy (r= 0.382) as in figure-1, and highly significant (p<0.001) relationship between HSAP and the gestational age during pregnancy (r= 0.382) as in figure -2.

A highly significant (p<0.001) relationship between HLAP and HSAP during pregnancy (r= 0.468) appear as in figure -3 .Relationship between serum PTH.log and HSAP during pregnancy was slightly significant (r= 0.337, n= 36, p < 0.045) as in figure -4.

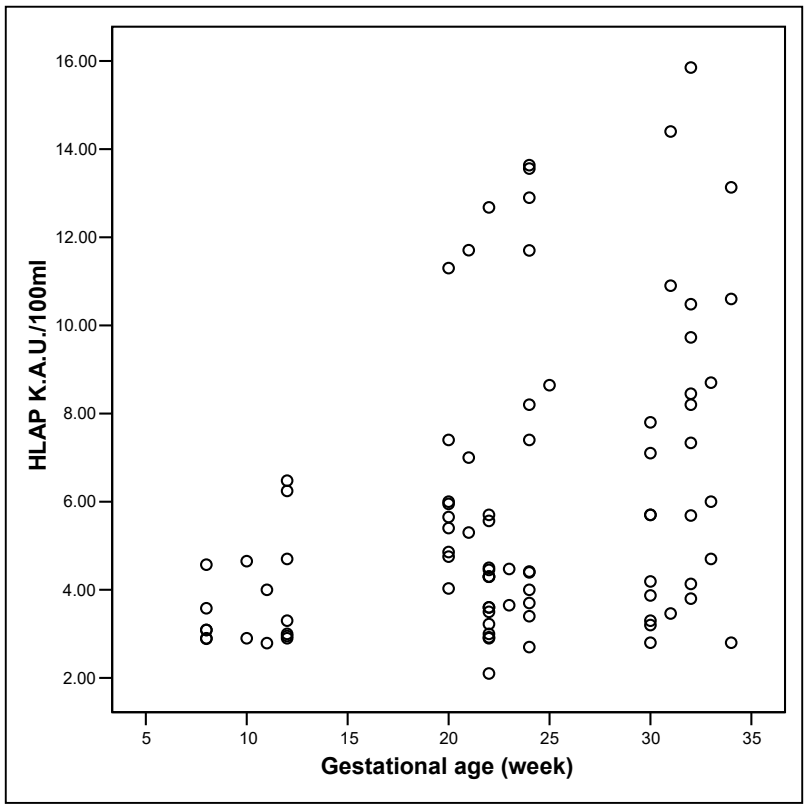

Figure -1: Relationship between serum HLAP and gestational age during pregnancy (r= 0.382, n= 87, p < 0.001)

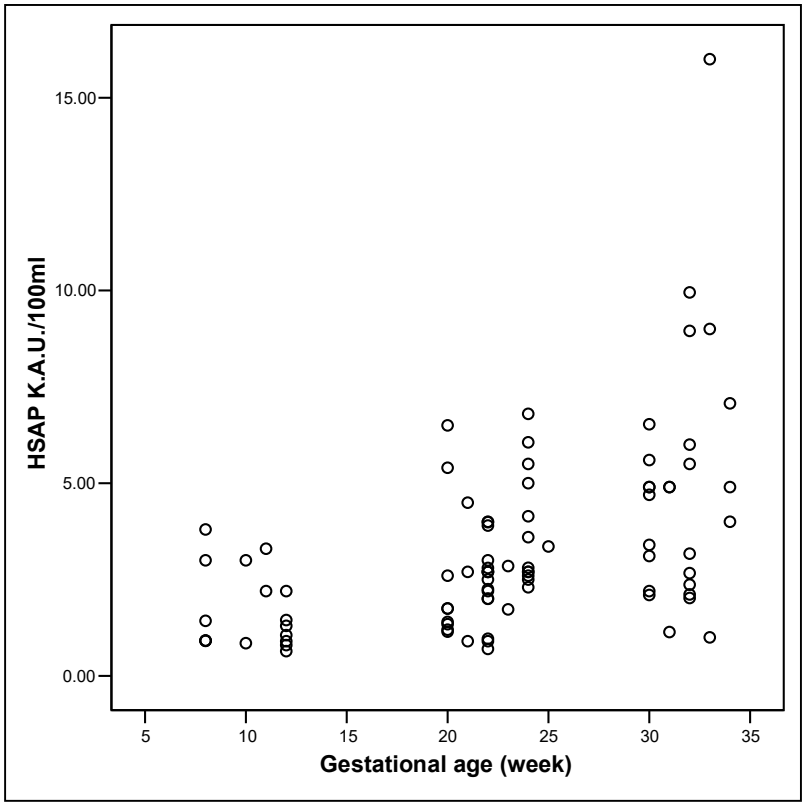

Figure -2: Relationship between serum HSAP and gestational age during pregnancy (r= 0.382, n= 87, p <0.001)

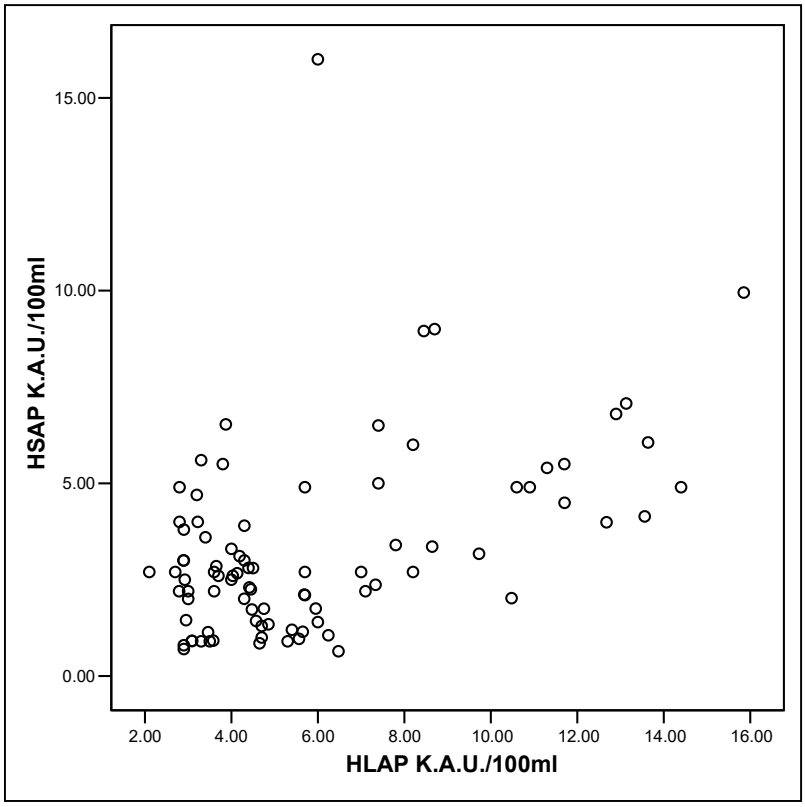

Figure -3: Relationships between serum HLAP and HSAP during pregnancy (r= 0.468, n= 87, p < 0.001)

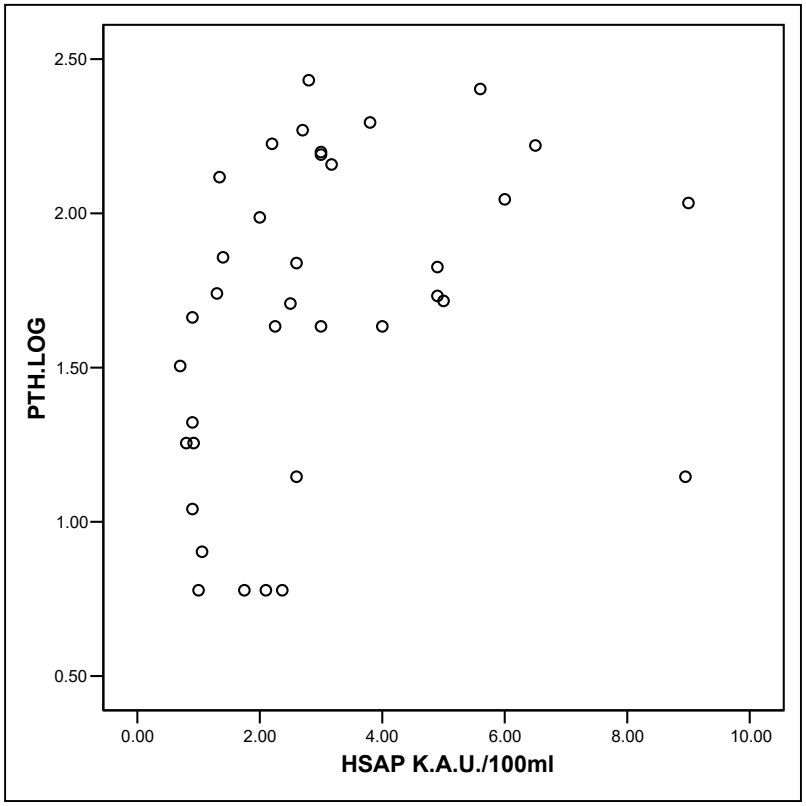

Figure -4: Relationship between serum PTH.log and HSAP during pregnancy (r= 0.337, n= 36, p < 0.045)

From figure - 5 we can clearly observed that HLAP mean values increase as the period of pregnancy increases, as the rate of these values was 3.76 , 5.97 , and 7.11 during 1st, 2nd, and 3rd trimester respectively. Also from figure - 6, we can see that the HSAP mean values increase with the increase in the gestation period, as the mean of these values was 1.69, 2.91, and 4.92 during the first, second and third trimester, respectively.

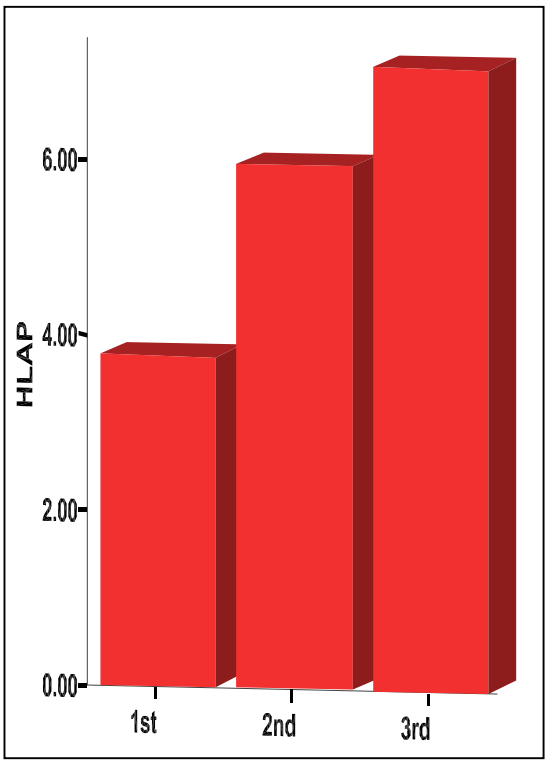

Figure -5: The mean of serum HLAP in the 1st, 2nd, 3rd trimester

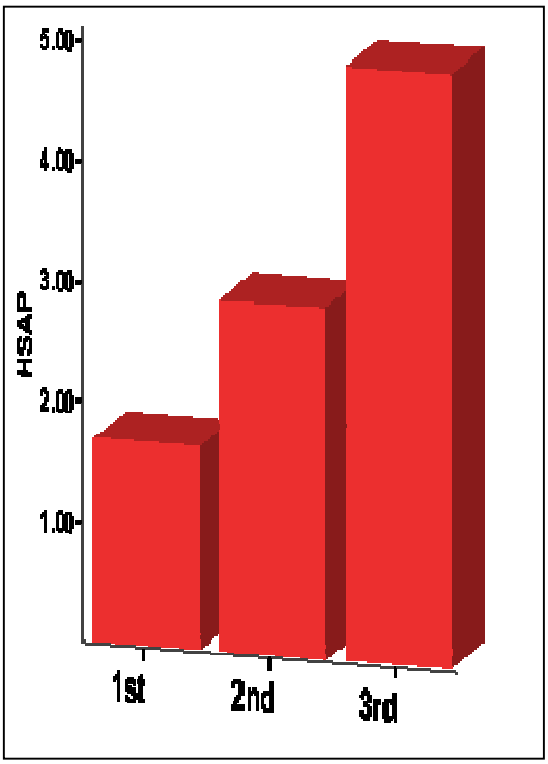

Figure -6: The mean of serum HSAP in the 1st, 2nd, 3rd trimester

DISCUSSION

The current study showed that there is a highly significant relationship ($p<0.001$) between HSAP and the gestational age during pregnancy, and also the difference between 1st and 2nd trimester, ($p<0.004$) and between 2nd and 3rd ($p<0.005$) is slight significant and the result between 1st and 3rd became highly significant ($p< 0.001$), all that Indicate the progressive increases in the level of placental isoenzyme during pregnancy, Reflecting the optimum function of placenta to supply fetal demand.

Also there is a highly significant relationship between HLAP and gestational age, and there is a highly significant difference between 1st and 2nd trimester ($p<0.001$) and between 1st and 3rd ($p<0.001$), and this progressive increase is proportionate with HSAP directly and significantly ($p<0.001$) indicating the increasing of bone resorption as response to placental growth and fetal demand i.e. biochemical osteomalacia.

There is apparently an increasing in PTH in our study relative to the general mean, of non-pregnant women that is the mean of PTH level in our study was (80.52 Pg. /mL) and its range between (6 – 270 Pg. /mL), and anti-PTH logarithm was (46.77 Pg. /mL) and its range was (6.03 – 269.15 Pg. /mL), while it is approximately (28.1Pg. /mL) in non-pregnant women aged (16–46) years according to (17), and 24.8 ± 9.0 ng/L in 11 normally cycling non pregnant women (10).

If we take into consideration the results of some studies indicate that either there is no significant elevation in PTH level and no significant difference in 25(OH)D (Which indicates the the storage of vitamin D in the body) during pregnancy (18), or PTH levels are significantly lower during pregnancy as compared to non-pregnant state (19, 20) that is mean the pregnant women in our study have pathological secondary hyperparathyroidism ,could be due to the biochemical osteomalacia.

Similar to our result a study found a Biochemical Osteomalacia (high HLAP) that present in Subjects had

higher PTH than did Women with normal HLAP (21), and The slightly significant (p<0 045) relationship between anti PTH.log and HSAP support this conclusion. This relationship perhaps become more significant if we take larger number of result, thus it is reflecting the increase in calcium and phosphorous transfer from mother to fetus through placenta via an active mechanism (8, 9) that lead to increase PTH as response to vitamin D deficiency where Serum PTH is inversely correlated with serum 25(OH)D (22).

CONCLUSION and RECOMMENDATION:

The elevated HLAP during pregnancy indicates a biochemical osteomalacia, and the secondary hyperparathyroidism is pathological and not physiological. The researchers recommends taking a measurement of parathyroid hormone and placental and bone ALP in pregnant women to ensure the health of the placenta and bone.

REFERENCES

1- Alonso, AGL, 2006, Effect of pregnancy on pre-existing liver disease physiological changes during pregnancy, J.Ann.Hep. 5 (3): 184-186 .

2- Prentice, A, Laskey, MA, Shaw, J, Hudson, GJ, Day, KC, Jarjou, L M A, Dibba, B, and Paul, AA , 1993, The calcium and phosphorus intakes of rural Gambian women during pregnancy and lactation, Brit.J.Nut. 69(3): 885-896.

3- Ujjawal Sharma, Deeksha Pal, and Rajendra Prasadcorresponding, 2014, Alkaline phosphatase: an overview. Indian J Clin Biochem. , 29(3): 269-278.

4- Burtis, C.A. and Ashwood, E.R., 2001, Fundamental of Clinical Chemistry. 5th Edition, W.B. Saunders Company, Philadelphia, London, St Louis, Sydney, Toronto, 1815p.

5- Deepak Joshi, Andra James, Alberto Quaglia, Rachel H Westbrook, Michael A Heneghan, 2010, Hepatic disorders in pregnancy are rare,(Liver disease in pregnancy), Lancet; 375(9714): 594–605.

6- Soleiman Mahjoub, Jila Masrour Roudsari , 2012, Quantification of

Liver Alkaline Phosphatase Isoenzyme Activity Using Heat Inactivation and Phenylalanine Inhibition Techniques: Comparison of Two Methods, World Applied Sciences Journal 17(8): 941-946.

7- Judkins, A, and Eagleton, C, (2006) Vitamin D deficiency in pregnant New Zealand women, The New Zealand med.j. 119(1241): 1175-8716.

8- Lobel BL, Deane HW and Romney S L., 1962, Enzymic Histochemistry of the Villous Portion of the Human Placenta from six weeks of Gestation to Term. American Journal of Obstetrics and Gynecology; 83(3): 295-299.

9- Catarina Tivane, Marcio Nogueira Rodrigues, Phelipe Oliveira Favaron, Antonio Chaves de Assis Neto, Eduardo Harry Birgel Júnior, Maria Angelica Miglino, 2013, Mechanisms of calcium transport across the placenta: Review , Open Journal of Animal Sciences , 3(1): 13-20.

10- Møller UK, Streym S, Mosekilde L, Heickendorff L, Flyvbjerg A, Frystyk J, et al. Changes in calcitropic hormones, bone markers and insulin-like growth factor I (IGF-I) during pregnancy and postpartum: A controlled cohort study, 2013, Osteoporos Int. , 24(4): 1307–1320.

11- Simmonds CS, Karsenty G, Karaplis AC, Kovacs CS. Parathyroid hormone regulates fetal-placental mineral homeostasis, 2010, J Bone Miner Res., 25(3): 594–605.

12- Chang, JM, Hwang, SJ, Tsai, JC, and Lai, YH, 2000, In vivo effect of endothelin-1 on plasma calcium and parathyroid hormone concentrations, J.End. 165(2): 179-184 .

13- Scantibodies (a), 2007, Rat total intact PTH ELISA kit enzyme-linked immunosorbent assay (ELISA), Part Number: 3KG024, Scantibodies Laboratory, Inc., 8p.

14- Ola Hysaj, Patricia Marqués-Gallego, Aline Richard, Magdeldin Elgizouli, Alexandra Nieters, Katharina Quack Loetscher, Sabine Rohrmann, 2021, Parathyroid Hormone in Pregnancy: Vitamin D and Other Determinants, Nutrients 13(2): 360-375.

15- Abdurrahman Avar Ozdemir, Yasemin Ercan Gündemir, Mustafa Küçük, Deniz Yıldıran Sarıca, Yusuf Elgörmüş, Yakup Çağ, Günal Bilek, 2018, Vitamin D Deficiency in Pregnant Women

and Their Infants, J Clin Res Pediatr Endocrinol, 10(1): 44-50.

16- Bland, M., 2003, An introduction to medical statistics, 3ed , Oxford University Press Inc., USA. 446P.

17- Zechariah Wilson Cecilia Lau Fred Caporaso Ken Sumida Frank Frisch, 2010, ALP to PTH Ratio in Young and Old Females, The FASEB Journal ,24(S1): 630-639.

18- More, S, 2007, Bone metabolism during pregnancy and lactation, Ph.D. Thesis, University of Debrecen, Medical and health science center, Department of obstetrics and gynecology, Prague, 17p.

19- J. B. Sharma, Subhadra Sharma, B. R. Usha, Manisha Yadav, Sunesh Kumar, and A. K. Mukhopadhyay, 2016, Cross-sectional study of serum parathyroid hormone level in high-risk pregnancies as compared to nonpregnant control, Indian J Endocrinol Metab., 20(1): 92–96.

20- Cyrus Cooper, Sarah Westlake, Nicholas Harvey, Kassim Javaid, Elaine Dennison, Mark Hanson, 2006, Review: developmental origins of osteoporotic fracture, Osteoporos Int , 17(3): 337-347.

21- Sachan, A, Gupta, R, Das, V, Agarwal, A, Awasthi, PK, and Bhatia, V , ,2005, High prevalence of vitamin D deficiency among pregnant women and their newborns in northern India, Am.J.Clin.Nutr. 81(5): 1060- 1064.

22- A. J. Sai, R. W. Walters, X. Fang, and J. C. Gallagher, 2011, Relationship between Vitamin D, Parathyroid Hormone, and Bone Health, J Clin Endocrinol Metab, 96(3): E436–E446.