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Biochemistry Section

Comparison of Serum C-reactive Protein, Parathyroid Hormone, and Calcitonin Levels between Pregnant and Non Pregnant Women from Rural North Gujarat: A Case-control Study

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ABSTRACT

Introduction: Pregnancy requires women to provide calcium to foetus in amounts that may exceed their daily intake. C-Reactive Protein (CRP) is a susceptible marker of systemic inflammation. Parathyroid hormone (PTH) regulates foeto-placental mineral homeostasis and skeletal development and stimulates placental calcium transfer. The increase in calcitonin and calcitriol levels are significant in the transport of maternal calcium to the foetus and in the anticipation, and revival of maternal bone loss. These changes have direct implications on calcium metabolism and cause decreased albumin level, inflammation, increase in extracellular fluid volume, increase in renal function, and placental calcium transfer.

Aim: To assess the serum levels of CRP, calcitonin, and PTH in the first, second, and third trimester of pregnancy and also to compare these parameters with non pregnant women.

Materials and Methods: The present case-control study was conducted in the Department of Biochemistry of Nootan Medical College and Research Centre, Visnagar, Gujarat, from June 2021 to October 2021. A total of 150 subjects (75 age and sex-matched apparently healthy well-nourished non pregnant females, and 75 pregnant females) were included. The CRP, PTH, and calcitonin were measured. Statistical analysis was performed using Independent t-test, and Pearson's correlation coefficient (r).

Results: Mean age of participants in case group was 32.16±10.97 (mean±SD). The mean age of control was 30.54±6.63. Serum CRP level of cases was significantly higher 3.2±2.2 than the level in controls 2.3±1.8 (p=0.003). Serum PTH level of cases 31.6±10.4 was significantly lower (p=0.0012) than the level in controls 45.9±9.8. Serum calcitonin level of cases 281±143 was significantly higher (p<0.001) than the level in controls 103±46.

Conclusion: There was a significant increase in serum CRP level and serum calcitonin level in cases as compared with controls, while there is decrease in serum PTH levels between these two groups.

Keywords: Inflammation, Maternal calcium, Placental transfer, Trimester

INTRODUCTION

Numerous biochemical and metabolic modifications occur during pregnancy. During pregnancy, women provides calcium to the foetus in amounts that may exceed their daily intake [1]. As a result, hormones involved in calcium homeostasis work together to congregate the mineral requirements [2]. In order to counteract the increased intestinal absorption of calcium, the requirement for calcitriol is like-wise raised [3]. It helps to sustain maternal calcium homeostasis [4]. High CRP level in pregnancy can be caused by a variety of inflammatory and infectious diseases [5].

The CRP is a susceptible marker of systemic inflammation. CRP accompanies both acute and chronic inflammatory disorders [6]. Serum concentrations of CRP in pregnancy are greater beyond non pregnant standards, and the variation being detected as early as four weeks gestation [7]. Although the direct synthesis of CRP by trophoblast may play a role, the specific aetiology of this increase is uncertain. Elevated concentrations of CRP in the 1st trimester have been related with preterm delivery [7]. The CRP levels in pregnant women who have preterm labour and early rupture of membranes have been calculated as a tool for identifying subclinical infection [7].

The PTH is an exceptionally essential hormone in calcium homeostasis [8]. It has a short half-life of five minutes and is prejudiced by slight changes in serum calcium levels. The need for calcium increases during pregnancy [9]. Maternal PTH levels are optimistically allied with birth weight, foetal upper arm, and calf circumferences [10]. The PTH is responsible for the foeto-placental mineral homeostasis, skeletal development, and stimulates placental calcium transfer [11].

Calcitonin stimulate renal 1,25 dihydroxy vitamin D 1,25(OH)2 {2 to be subscribed} production in the proximal tubule [12]. During pregnancy and lactation, both calcitonin and calcitriol are increased. Elevated calcitonin and calcitriol levels may have a role in the delivery of maternal calcium to the foetus/infant, as well as the prevention and restoration of maternal bone loss [13].

Calcitonin is the hypocalcemic, hypophosphatemic polypeptide of C-cell origin, and it has been reported to increase in pregnant women at the time of delivery. In a study, high levels of this hormone was determined by radioimmunoassay in pregnant women in all trimesters [14]. Several studies have found that levels were also higher for the first two days after delivery [14,15]. Few researches reported, that that except in the first trimester, when the calcitonin hormone's level was inversely related to blood phosphate, calcitonin levels were not linked to serum calcium or phosphate [14,15]. Possibly, pregnancy-induced hypercalcitonaemia protects the mother's skeleton while allowing the foetus to accumulate calcium [14].

During the course of the pregnancy, an extraordinary series of physiologic changes occur, intended at preserving maternal homeostasis while at the same time providing for foetal growth and development [16]. Most of the studies mainly focussed to measure the parameters in normal pregnancy and to associate them with some risk factors [17,18]. Hence, present study was conducted to measure the CRP, PTH, and calcitonin levels in all the three trimesters, and also compared their levels with non pregnant women.

MATERIALS AND METHODS

The case-control study was conducted in the Department of Biochemistry of Nootan Medical College and Research Centre, Visnagar, Gujarat, from June 2021 to October 2021. Ethical clearance was obtained by Institution's Human Research Ethics Committee. (Ref: IEC/NMCRCAPPROVAL/44/2021)

Inclusion criteria: Women aged between 21-45 years, recalling the exact date of the last menstrual period, whose gestational age was appropriate according to trimester in the first visit, and BMI ranging between 26 to 30 kg/m^2 were included in the study. Age-matched, non pregnant, and healthy controls were recruited from those who reported for the health check-up scheme of the hospital.

Exclusion criteria: Participants having any systemic disease, endocrine disorder or infections and any history of complicated pregnancy were excluded.

Sample size calculation: Sample size was calculated using Cochran's formula;

Sample size=$Z^2 \times SD^2/Precision^2$

Power of study was 80% [19]. Using SD and Precision of other studies on pregnant woman [20,21], sample size arrived was 40 and 59, respectively.

Altogether 150 participants were enrolled in the present study. A total of 75 were recruited as case and 75 as controls.

In the case group, participants were divided into 1st, 2nd and 3rd trimesters with 25 participants in each trimesters. In the control group (n=75), participant were divided into three groups of 25 participants each, where each control group was compared with individual case group. Age and sex were matched for all three groups.

Study Procedure

Five mL of blood samples were collected and allowed to clot, and then centrifuged at 3400 RPM for 15 min at 20-22°C to obtain the serum. Parameters like serum CRP, PTH, and calcitonin were evaluated and compared between the cases and controls.

Serum CRP was measured by antigen-antibody reaction by the end-point method using Erba EM 200 fully auto analyser. PTH concentration in human serum was determined using an immunoenzymatic colorimetric technique. Calcitonin concentration in human serum was determined using an immunoenzymatic colorimetric technique. Serum PTH and serum calcitonin were analysed using Enzyme-linked Immunosorbent Assay (ELISA) machine. Reference range for CRP is 0-10 mg/L, [22] for PTH 9-94 pg/mL, [23] and for calcitonin is <11 pg/mL [24].

STATISTICAL ANALYSIS

Analysis was performed using the commercially available Statistical Software STATA (14.2), and Microsoft Excel 2016. The p-value of less than 0.05 was considered statistically significant. All of the parameters were analysed by applying Independent t-test and Pearson's correlation coefficient (r).

RESULTS

Mean age of participants in case group was 32.16±10.97 years and that in the control group was 30.54±6.63 yrs. The mean BMI for cases and controls were 31.66±1.5 kg/m^2 and 23.88±1.8 kg/m^2, respectively [Table/Fig-1].

Variables	Cases (75)	Controls (75)	p-value
Age (years)	32.16±10.97	30.54±6.63	0.2755
BMI (kg/m^2)	31.66±1.5	23.88±1.8	<0.001

[Table/Fig-1]: Baseline data of participants.
Independent t-test, level of significant p-value <0.05; Values presented as mean±SD

Serum CRP levels of 1st, 2nd, and 3rd trimesters were 2.5±1.9 mg/L, 3.1±2.1 mg/L, and 3.8±2.4 mg/L, respectively. Serum PTH levels of 1st, 2nd, and 3rd trimesters were 35.5±11.1 pg/mL, 25.5±8.3 pg/mL, and 32.4±10.2 pg/mL, respectively. Serum calcitonin levels of 1st, 2nd, and 3rd trimesters were 225±106 pg/mL, 343±181 pg/mL, and 306±143 pg/mL, respectively [Table/Fig-2].

Parameters	First trimester (1)	Second trimester (2)	Third trimester (3)	p-value
Gestational age (weeks)	10±2	21±4	38±3	0.05
Serum CRP (mg/L)	2.5±1.9	3.1±2.1	3.8±2.4	1 vs. 2=0.2947
				2 vs. 3=0.2779
				1 vs. 3=0.0389
Calcitonin (pg/mL)	225±106	343±181	306±143	1 vs. 2=0.0071
				2 vs. 3=0.4265
				1 vs. 3=0.0274
PTH (pg/mL)	35.5±11.1	25.5± 8.3	32.4±10.2	1 vs. 2=0.0007
				2 vs. 3=0.0116
				1 vs. 3=0.3090

[Table/Fig-2]: Comparison of CRP, PTH, and calcitonin among trimesters.
Independent t-test, level of significant p-value <0.05

Serum CRP levels of cases was significantly higher 3.2±2.2 than the level in controls 2.3±1.8 (p=0.003). Serum PTH level of cases 31.6±10.4 was significantly lower (p=0.0012) than the level in controls 45.9±9.8. Serum calcitonin level of cases 281±143 was significantly higher (p<0.001) than the level in controls 103±46 [Table/Fig-3].

Parameters	Case	Control	p-value
Serum CRP (mg/L)	3.2±2.2	2.3±1.8	0.003
Calcitonin (pg/mL)	281±143	103±46	0.0012
PTH (pg/mL)	31.6±10.4	45.9±9.8	<0.001

[Table/Fig-3]: Serum CRP, PTH, and calcitonin levels in pregnant and non pregnant women.
Independent t-test, level of significant p-value <0.05

The CRP level had shown increment as 2.9 mg/L, 3.1 mg/L, 3.4 mg/L, 3.6 mg/L, 3.7 mg/L, 3.7 mg/L, 3.9 mg/L, and 4.3 mg/L according to their gestational age of 16, 20, 24, 28, 32, 36, 38, and 40 weeks, respectively [Table/Fig-4]. There was a significant positive correlation between CRP levels and gestational age (r=0.827, p=0.00015) [Table/Fig-4]. Authors had established significant but weak correlation between PTH level and gestational age (r=0.114, p=0.0118). Authors had established significant positive correlation between calcitonin level and gestational age (r=0.628, p=0.000016) [Table/Fig-4].

Week of gestation	CRP (mg/L) [mean±SD]	Calcitonin (pg/mL) [mean±SD]	PTH (pg/mL) [mean±SD]
16	2.9±2.5	332±178	22.5±8.1
20	3.1±2.4	340±181	25.7±8.2
24	3.4±2.4	358±184	28.9±8.4
28	3.6±2.3	315±146	29.6±9.9
32	3.7±2.5	312±145	30.9±10.2
36	3.7±2.5	306±142	32.4±10.1
38	3.9±2.8	301±140	33.5±10.3
40	4.3±2.5	295±138	35.4±10.4

Mean value	3.2±2.2	225±106	31.6±10.4
p-value	0.00015	0.000016	0.0118
r value	0.827	0.114	0.628

[Table/Fig-4]: Serum CRP, PTH, and calcitonin levels in pregnant women and their correlation with gestational age.
Pearsons correlation, level of significant p-value <0.05

DISCUSSION

In the present study, a significant increase in the level of serum CRP (3.2±2.2 mg/L) and calcitonin level (281±143 pg/mL) in cases was found, as compared with controls (2.3±1.8 mg/L, 103±46 pg/mL respectively); while there was a decrease in serum PTH level between cases and controls.

Sacks GP et al., provides evidence for maternal inflammatory response with elevated CRP levels as early as four weeks gestation [25]. Rebelo I et al., reported that the CRP levels are raised later in the 1st trimester [26] and Teran E et al., reported raised CRP levels later in the 3rd trimester [27]. Higher concentration of CRP has been found during 2nd and 3rd trimester in present study. Sacks GP et al., proposed the occurrence of a systemic maternal inflammatory response in the 3rd trimester, which has been widely documented [28]. In normal pregnancy, there is evidence of extensive initiation of maternal immune system innate components and these changes comprise the maternal inflammatory response [28].

In a previous cross-sectional investigation employing an identical method for measuring PTH, cases had lower intact PTH concentrations than controls, which supports the findings of the current study [29]. Davis OK et al., proposed that vitamin D is the primary hormone responsible for maintaining maternal calcium homeostasis throughout pregnancy [29]. Vitamin D levels arise during pregnancy [30]. Interestingly, previous studies showed a weak positive correlation between PTH level and gestational age [31,32]. Serum PTH levels fell to the 10-30% of the mean non pregnant value and then increased progressively to a mid-normal range by the term [31,32], which is consistent with the finding of the present study [31,32]. The mean concentrations of intact PTH in late pregnancy were lower than in non pregnant women, according to Kaneshapillai A et al., which would support the current study [32].

Konopka P et al., measured the serum calcitonin of non pregnant and pregnant women using bioassay and observed that 57.4% of pregnant women had elavated values [15]. In present study, 70% of pregnant women had elevated values of calcitonin. These had concluded some congruent results supporting with present study. The amplification during gestation was statistically noteworthy in the 2nd and 3rd trimester. These authors proposed that hypercalcitonemia may serve to defend the skeleton against demineralisation during pregnancy. Samaan NA et al., initially reported that iCT levels were high in women at delivery that would in support of present study [33].

Some authors were not in agreement with the present study results, when they found no correlation between serum calcitonin and pregnancy [34]. Pitkin RM et al., found no steady change in iCT during a longitudinal study of pregnant subjects [34]. Pregnancy exerts a intense influence upon calcium metabolism, accompanied as it is by osseous formation in the foetus. The human foetus accumulates 20-30 gm of calcium, mostly in the 3rd trimester. Duggin GG et al., found that women have a positive calcium balance during pregnancy, but considerably less so than the amount estimated to be necessary for the foetus [35]. The increased levels of iCT during gestation propose that this hormone plays a role in the defense of the maternal skeleton.

The present study was conducted in rural north Gujarat population, which revealed alteration of biochemical marker in pregnancy. Further studies which can reflect correlation of inflammatory markers and calcium homeostasis, will illuminate study more deeply.

Limitation(s)

The CRP measurement range was excessively broad, which could lower the study's importance and lead to bias. Another drawback is that CRP is a relatively generic marker that despite tight exclusion criteria, might be positive or high for other reasons, resulting in bias.

CONCLUSION(S)

In the present study, it was found that there was a significant increase in serum CRP level in pregnant women as compared with normal subjects and there was a significant increase in serum calcitonin in pregnant women as compare with normal subjects, while there was decrease in serum PTH level in the cases as compared to control group. In conclusion, regarding the above mentioned studies, it seems reasonable to perform further studies for determining the predictive value of serum CRP and to try to find an optimum cut-off point. Further studies which can reflect correlation of inflammatory markers and calcium homeostasis will illuminate study more deeply. Further studies are warranted to widen the knowledge about pathophysiological mechanisms linking inflammation and pregnancy complications. More inflammatory and calcium homeostasis markers can be added in future.

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