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Assessment of Relationship Between Calcium-Phosphorus Ratio And Parathyroid Hormone Levels In Serum of Osteoarthritic Disordered Patients: A Diagnostic Protocol

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Abstract
Introduction: *Osteoarthritic disorders (OADs) are degenerative and painful diseases, which found worldwide, also in India. The present study was aimed to determine an association between calcium-phosphorus ratio and parathyroid hormone levels in the serum of patients having OADs.*
Methods: *In the present study, a total 100 patients, aged (40 -≤ 80) years old (female: 73 and male: 27) having OADs in right and/or left knee joints, hip joints, ankle joints and degenerative changes in cervical and lumbar vertebrae as experimental subjects and 100 subjects with same age group (female:male = 73:27) having no OADs in any joint of the body as control subjects were considered. 5ml of peripheral blood samples were taken from each subject of both the groups and serum was used for the analysis of calcium, phosphorus, calcium-phosphorus ratio and intact-parathyroid hormone levels.*
Results: *The results were obtained a decreasing level of calcium and phosphorus in the experimental group of combined, female and male subjects compared to that of control group without any significant changes (combined subjects: P=0.16; female: P=0.39; male: P=0.17 for calcium and combined subjects: P=0.02; female: P=0.03; male: P=0.07 for phosphorus). All the data for the calcium-phosphorus ratio of the experimental group of combined, female and male subjects were at significant levels (P<0.01; P<0.05 and P<0.05) in comparison with that of the control group. In the case of i-PTH levels in serum of subjects were shown an increasing trend in the experimental group of combined, female and male subjects at significant levels (P<0.001; P<0.01 and P<0.01) when compared that to control group. The comparative study of each level of calcium, phosphorus, calcium-phosphorus ratio with the i-PTH level in serum of experimental subjects showed lower calcium-phosphorus ratio followed by phosphorus while little higher calcium level but an increasing trend in the i-PTH level in comparison with control group. The comparison results were highly significant between calcium and i-PTH levels (F value = 53.402, P = 0.000), phosphorus and i-PTH levels (F value = 90.261, P = 0.000) and calcium-phosphorus ratio and i-PTH levels (F value = 97.572, P = 0.000) in the serum of experimental subjects.*
Conclusions: *The hypothesis from results showed lower calcium-phosphorus ratio and higher i-PTH levels, which may be a suitable diagnostic protocol to assay OADs besides radiological and MRI imaging.*
Key words: *Calcium-phosphorus ratio and parathyroid hormone, Osteoarthritic disorders and diagnostic tool, Hypocalcaemia, Hypophosphatemia, Hyperparathyroidism*

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I. Introduction

Osteoarthritic disorders (OADs) are a bone degeneration and inflammatory disease [1-5]. This disease causes in both knee joints, ankles, hip joint and vertebral column, etc. and several diagnostic protocols on the basis of radiology, anatomy, biochemistry, hematology, etc. have already been established [8-12]. Several biochemical parameters have altered abnormally in various tissues viz. blood, cartilages, bones and synovial fluid during osteoarthritis in male and female adults [12,13-19]. Besides, above-mentioned biochemical biomarkers, the calcium/phosphorus ratio in the blood is a very important biomarker during bone formation [20]. Generally, any diseases lead to oxidative stress and found free radical generation in different tissues. It was documented that inflammation and rheumatoid arthritis causes oxidative stress and the level of calcium/phosphorus decreases in blood [20,21-22]. In other words, calcium and phosphorus are known as micronutrients. Among these, two elements, calcium supports vascular contraction, vasodilation, glandular secretion, muscular contraction, glycogen metabolism, neurotransmission and finally maintain bone health and mineralization [23-24] and phosphorus enhances the metabolism of minerals, signal transduction in cells,

exchange of energy and along with calcium helps in bone development [24-25]. Calcium is tied up with several anions such as phosphate, bicarbonate, and citrate. It has already been established that lowering calcium levels in serum leads to bone deformation, renal diseases, hypoparathyroidism etc. [26-27]. According to Thomas [26] and Endres and Rude [27], increased total calcium can be estimated in diseases like hyperparathyroidism, etc. The phosphorus is found in serum that important inorganic mineral of bones and associated strongly with calcium level [26-27]. Likewise, parathyroid hormone (PTH) is secreted by the parathyroid glands. Parathyroid hormone (PTH) exerts a major influence over the systemic rate of bone resorption, which helps by osteoclast (bone reabsorbing cells). The PTH injection leads to rapid induction of the activity of pre-existing osteoclasts, also supported by an enhancement of osteoclast counts [28-29]. The researchers have documented that PTH increases the resorptive activity of pre-existing osteoclasts through a primary hormonal interaction with cells of the osteoblastic lineage, which possess PTH receptors and responsiveness [30-32]. Fuller et al. [32] have stated that there is a close relationship and also a dependency for osteoclast formation of a direct action of $1,25(OH)_2D_3$ and/or PTH on osteoclastic precursors. It was reported that declining calcium in the blood stimulates secretion of PTH. According to Moe [33], the function of PTH is basically maintaining calcium homeostasis through a mechanism by increasing bone mineral dissolution, which helps in releasing calcium and phosphorus, inducing renal calcium reabsorption and phosphorus excretion, and finally increasing the gastrointestinal absorption of both calcium and phosphorus indirectly through its effects on the synthesis of 1,25 $(OH)_2D$ or 1,25-dihydroxy vitamin D_3, known as calcitriol. On the other hand, calcitriol plays an important role to maintain the calcium-phosphorus level in the peripheral blood and bone mineralization can easily be occurred within the body of a human. An established mechanism found excess PTH secretion leads to lowering the blood calcium-phosphorus ratio during several diseases viz. bone and cartilage deformities, skin, neurological disorders, chronic renal disorders and several systemic manifestations [33-35]. Excess PTH secretion in the blood causes hypocalcemia, hyperphosphatemia, and calcitriol deficiency [33]. The 1α-hydroxylase (CYP27B1) in the kidney is regulated by nearly all hormone involved in calcium homeostasis, and its activity is stimulated by PTH, estrogen, calcitonin, prolactin, growth hormone, low calcium and low phosphorus [33]. The calcium homeostasis is inhibited by calcitriol, which is providing the 'feedback' loop, ultimately modulates calcitriol synthesis. The most important function is exhibited in the small intestine, where calcitriol regulates the intestinal absorption of calcium and, to a lesser degree, phosphorus [36] and inhibits PTH synthesis at the parathyroid gland [33]. Several research works reveal in the association between lowering calcium-phosphorus level and diseases as well as PTH induction and disorders. Estimation of calcium-phosphorus and PTH level during various diseases viz. renal failure, heart diseases, neurological disorders, rheumatic arthritis, etc. have already been established [4-5,24,36-37]. But no one has been attempted a combined relationship between lower calcium-phosphorus ratio and higher PTH levels in the blood of OADs patients.

The present study was an attempt to assay an association between lower calcium-phosphorus ratio and higher parathyroid hormone levels in the peripheral blood of patients having osteoarthritic disorders.

II. Materials And Methods

2.1 Recruitment of patients

A total three hundred six patients, aged (40-≤ 80) years old, (female: 188 and male: 118) from OPTM Health Care (P) Ltd, Kolkata, Delhi and Mumbai centers, India from July 2016 to December 2016 were evaluated in this study. The study protocol was evaluated and approved by the OPTM Research Institute Ethics Committee. The Institute is Government registered. Approved Institutional Review Board consent form for physical examinations, blood samples collection and cervical spine, lumbar spine, hip joints and knee joints images (either X-rays or CT scan or MRI) required for the study were signed by all patients in the first phase of the screening procedure.

2.2 Exclusion criteria

106 patients (female: 42 and male: 64) out of 306 patients were excluded due to following reasons: a) patients with rheumatoid arthritis, b) with parallel multiple drug dependence, c.) with surgical implants, d) with pacemaker, e) with history of cancer, f) with cuts, wounds, or any type of chronic skin disease and g) with history of severe neurological diseases.

2.3 Study design

After the analysis of exclusion criteria, out of the balanced subjects of 200 nos., 100 combined subjects (female: 73 and male: 27) having no complaints of pain and visual inflammation and no sign of OADs either in cervical region, lumbar region, hip joints, knee joints or any parts of the body as evidenced by X-ray or CT scan or MRI reports considered as control subjects. The rest of 100 subjects (female: 73 and male: 27) having OADs in any regions jointly or singly such as the cervical spine or lumbar spine or hip joints or knee joints and complain of pain and visual inflammation as evidenced by X-ray or CT scan or MRI reports considered as experimental subjects group. The demographic characteristics of all patients at the baseline were recorded in Table 1 and few radiological images for OADs are exhibited (Fig 1). The blood samples were collected from both experimental subjects and control subjects to assay calcium, phosphorus, calcium-phosphorus ratio and i-PTH.

2.4 Specific biochemical parameters in serum

5 ml of blood sample were collected in a vial (vial containing EDTA) from each subject of experimental group and control group. Then blood samples were centrifuged at 1000×g for 10 min at 4^{o}C to obtain serum. Finally, the serum was used to analyze calcium, phosphorus and i-PTH hormone for both experimental and control subjects. The quantitative assessment of calcium (mg/dl) was carried out by using a kit Calcium AS FS by the method of the photometric test by using arsenazo III (DiaSys Diagnostic Systems Gmbh, Germany) at a wavelength of 650nm. The kit has developed from the methods of Michaylova and Ilkova [38] and Bauer [39].The quantitative assessment of phosphorus (mg/dl) was carried out by using a kit Phosphate FS by the method of photometric test (DiaSys Diagnostic Systems Gmbh, Germany) at a wavelength of 340nm. The kit has developed from the methods of Thomas [26]. The quantitative assessment of PTH (pg/ml) was carried out by using a kit Intact-PTH ELISA (Biomerica Inc., U.S.A, Ref 7022) as an immunoassay. The kit has developed from the methods of Raisz et al. [40], Mallette [41] and Kruger et al. [42].

2.5 Statistical analysis

Statistical analysis for control and experimental subjects was done by using student's 't' test and P values <0.05 were considered as statistically significant. The one-way ANOVA (analysis of variance) was also done by using the online ANOVA Calculator [43] for comparing three groups as combined, females and males for calcium and i-PTH, phosphorus and i-PTH as well as the calcium-phosphorus ratio and i-PTH level in the serum of OAD subjects. The statistical differences were considered through F value and P value in ANOVA.

Table 1: Demographic data and baseline characteristics of subjects

	Experimental Group			**Control Group**		
	Total	**Female**	**Male**	**Total**	**Female**	**Male**
No of Subjects	**100**	**73**	**27**	**100**	**73**	**27**
Age (years), mean (SD)	60.32 (9.73)	59.56 (8.94)	62.37 (11.35)	58.47 (9.03)	57.26 (8.27)	61.74 (10.13)
BMI (kg/m^2), mean (SD)	30.25 (7.49)	30.15 (7.13)	30.51 (8.37)	26.60 (6.92)	26.15 (7.13)	27.81 (6.19)
Period of Suffuring (years), mean (SD)	6.03 (4.75)	6.12 (4.13)	5.78 (6.12)	-	-	-
Ethnic Group (Indian Varities) (%)						
Bengali	31 (31%)	26 (35%)	5 (18%)	28 (28%)	23 (31%)	5 (19%)
Gujrati	10 (10%)	5 (7%)	5 (18%)	12 (12%)	8 (11%)	4 (15%)
Marwaree	12 (12%)	8 (11%)	4 (16%)	14 (14%)	9 (12%)	5 (19%)
Marathi	15 (15%)	11 (15%)	4 (16%)	15 (15%)	11 (15%)	4 (15%)
Tamil	10 (10%)	7 (10%)	3 (11%)	9 (9%)	6 (8%)	3 (11%)
Punjabi	9 (9%)	7 (10%)	2 (7%)	9 (9%)	7 (10%)	2 (7%)
Shindhi	7 (7%)	5 (7%)	2 (7%)	8 (8%)	6 (8%)	2 (7%)
North East India	6 (6%)	4 (5%)	2 (7%)	5 (5%)	3 (5%)	2 (7%)
Food Habit (%)						
Vagetarian	47 (47%)	34 (47%)	13 (48%)	51 (51%)	41 (56%)	10 (37%)
Non - Vagetarian	53 (53%)	39 (53%)	14 (52%)	49 (49%)	32 (44%)	17 (63%)
Multiple Complains (%)						
Constipation	35 (35%)	21 (29%)	14 (52%)	37 (37%)	28 (38%)	9 (33%)
Overweight / Obesity	36 (36%)	23 (31%)	13 (48%)	34 (34%)	26 (36%)	8 (30%)
Acidity & Refluse	27 (27%)	17 (23%)	10 (37%)	29 (29%)	22 (30%)	7 (26%)
Insomnia	30 (30%)	18 (25%)	12 (44%)	27 (27%)	21 (29%)	6 (22%)
Varicose Vein	28 (28%)	14 (19%)	14 (52%)	26 (26%)	19 (26%)	7 (26%)
Urinary Incontinence	26 (26%)	16 (22%)	10 (37%)	31 (31%)	27 (37%)	4 (15%)
Osteoarthritic Disorders (OADs) (nos)						
OA Changes in Rt Knee Joints	28	16	12	-	-	-
OA Changes in Lt Knee Joints	35	21	14	-	-	-
OA Changes in both Knee Joints	31	19	12	-	-	-
Degenerative Changes Cervical Region	27	15	12	-	-	-
Degenerative Changes Lumber Region	28	17	11	-	-	-
Degenerative Changes in Rt Hip Joints	15	9	6	-	-	-
Degenerative Changes in Lt Hip Joints	14	8	6	-	-	-
Degenerative Changes in both Hip Joints	13	8	5	-	-	-
Work Status						
Employed Fultime	20 (20%)	11 (15%)	9 (34%)	25 (25%)	17 (23%)	8 (30%)
Employed Part Time	16 (16%)	10 (13%)	6 (22%)	12 (12%)	8 (11%)	4 (15%)
House Wife / Home Maker	18 (18%)	18 (25%)	-	15 (15%)	15 (21%)	-
Retired	24 (24%)	18 (25%)	6 (22%)	22 (22%)	15 (21%)	7 (25%)
Self Employed	22 (22%)	16 (22%)	6 (22%)	26 (26%)	18 (24%)	8 (30%)

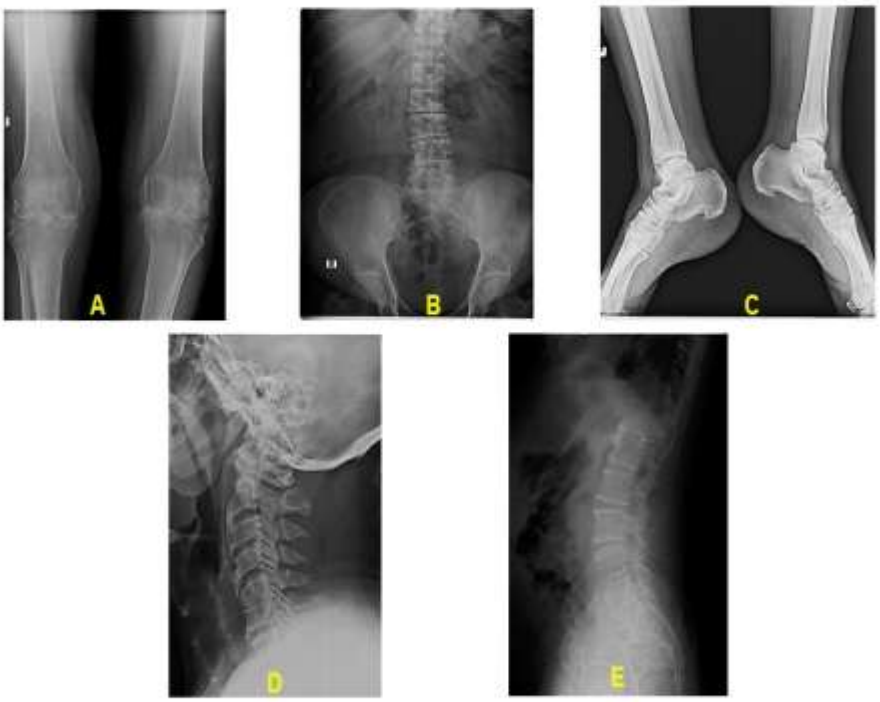

Fig. 1. Radiological images of OADs on bone degenerations (A = knee-joints, B = hip-joints, C = ankle-joints, D = cervical vertebrae, E = lumbar vertebrae**)**

III. Results

Different biochemical parameters with special references to calcium, phosphorus, calcium-phosphorus ratio and intact- parathyroid hormone were examined in the serum sample of experimental subjects suffering from OADs compared to control subjects having no OADs. The comparisons were made on the combined, female and male subjects separately for both the experimental group versus control group. As per Fig. 2, it was found that a decreasing level of calcium in the experimental group of combined subjects (9.93 ± 0.33 to 9.85 ± 0.46), females (9.92 ± 0.33 to 9.86 ± 0.49) and males (9.93 ± 0.33 to 9.85 ± 0.37), when compared with that of the control subjects. The differences were not shown any significant changes between the control and experimental groups (combined subjects: P = 0.16; female: P = 0.39; male: P = 0.17). Further, decreasing levels of phosphorus were also noticed in the OAD subjects separately with combined subjects (3.94 ± 0.45 to 3.76 ± 0.59), females (4.01 ± 0.45 to 3.82 ± 0.59) and males (3.78 ± 0.39 to 3.54 ± 0.55), when compared with that to control group. The differences of data between the control group and experimental group were not show any significant changes (combined subjects: P = 0.02; female: P = 0.03; male: P = 0.07) as exhibited in Fig. 3. In Fig. 4, the calculation was worked out for the calcium-phosphorus ratio of experimental subjects compared with control subjects. The ratio was found an increasing trend in the experimental group separately with combined subjects from 2.54± 0.27 to 2.70±0.49, females from 2.50± 0.25 to 2.65± 0.48 and males from 2.66 ±0.28 to 2.91 ±0.49 and all the data were at a significant level ($P < 0.01$; $P < 0.05$ and $P < 0.05$).

The levels of i-PTH in serum were also found an increasing trend in the experimental group separately with combined subjects from 20.83 ± 9.17 to 29.79 ± 17.55, females from 20.52 ± 9.61 to 28.07 ± 16.59 and males from 20.38 ± 6.57 to 34.54 ± 19.21, when compared with that to control group. All the data were within the significant levels ($P < 0.001$; $P < 0.01$ and $P < 0.01$) as shown in Fig. 5. As per Fig. 6, when the mean values for calcium, phosphorus, calcium-phosphorus ratio and i-PTH levels in serum of subjects of experimental group separately with combined subjects, females and males compared with that to control group, it was observed that lower calcium-phosphorus ratio followed by phosphorus while little higher calcium level, but an increasing trend in the i-PTH level for OAD subjects. The comparisons data were highly significant between calcium and i-PTH (F value = 53.402, P = 0.000), phosphorus and i-PTH (F value = 90.261, P = 0.000), calcium-phosphorus ratio and i-PTH (F value = 97.572, P = 0.000).

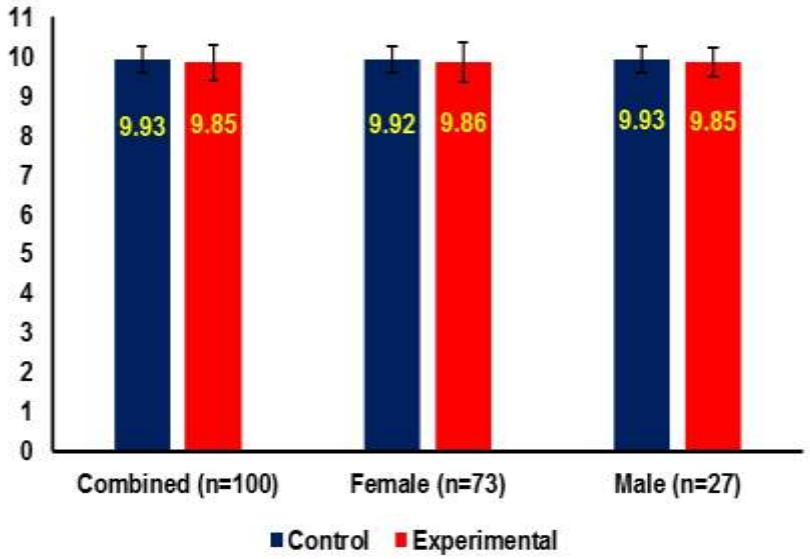

Fig 2: Calcium level in the blood of OAD patients (Combined P = 0.16; Female P = 0.39; Male P = 0.17)

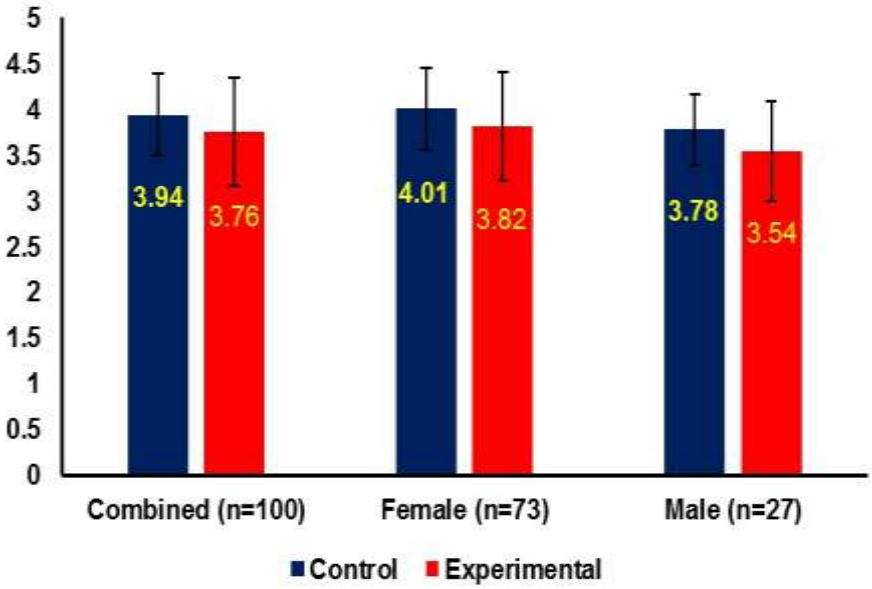

Fig 3: Phosphorus level in the blood of OAD patients (Combined P = 0.02; Female P = 0.03; Male P = 0.07)

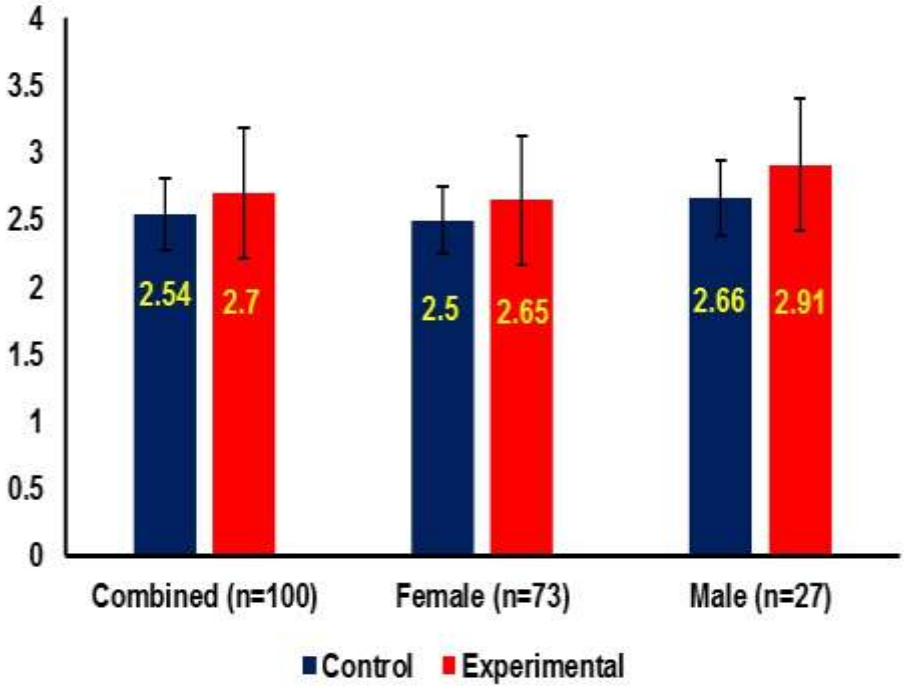

Fig 4: Calcium-phosphorus ratio in the blood of OAD patients (Combined P < 0.01; Female P < 0.05; Male P < 0.05)

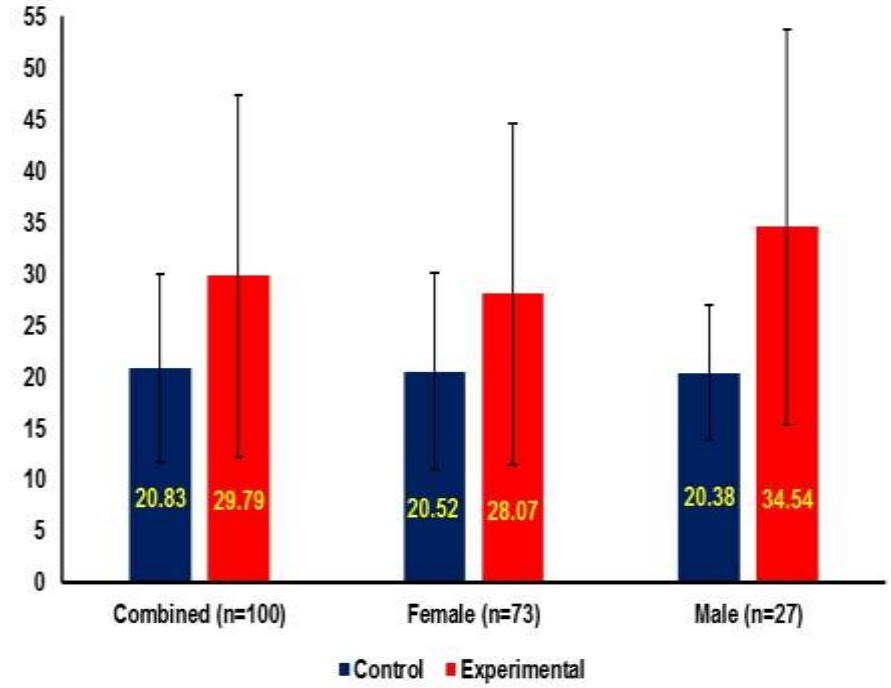

Fig 5: i-PTH level in the blood of OAD patients (Combined P < 0.001; Female P < 0.01; Male P < 0.01)

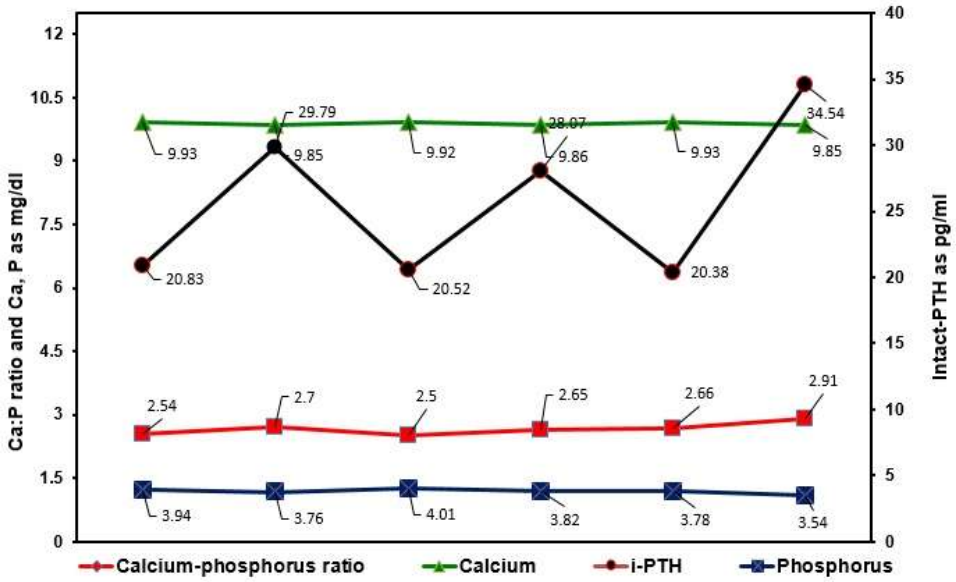

Fig 6: Comparison between calcium and i-PTH (F value = 53.402, P = 0.000), phosphorus and i-PTH (F value = 90.261, P = 0.000), calcium-phosphorus ratio and i-PTH (F value = 97.572, P = 0.000) level in the blood of OAD patients

IV. Discussion

The present study indicated the interpretation of blood biochemistry results with special reference to calcium, phosphorus, calcium-phosphorus ratio and intact- parathyroid hormone in the serum of OAD subjects of combined, females and males in comparison with that of control subjects. The blood parameters such as calcium and phosphorus, both were declined in experimental subjects for combined, females and males when compared with that to control subjects. All the data did not show any significant differences (Fig. 3 and 4). The reference ranges have been established of calcium between 8.6 – 10.3 mg/dl as per Endres and Rude [27] while phosphorus for adults between 2.6 – 4.5 mg/dl as per Thomas [26]. In the case of serum calcium level, the data observed at the minimal difference, which may emphasize the extra intake of calcium supplement during OADs and/or the habitual diet containing calcium viz. milk, yogurt, cheese, etc. [24]. Overall, declining of serum calcium and phosphorus has an evidence with bone disorders, especially bone joint pain, morphological deformities, abnormal bone mineralization, etc. [20-27]. The present results were indicated high serum calcium-phosphorus ratio significantly in the experimental subjects for combined ($P < 0.01$), females ($P < 0.05$) and males ($P < 0.05$) when compared with that of control subjects (Fig. 4). This may be due to the high calcium supplement and/or habitual diet during OADs [24].

There is a contradiction with present data on the calcium-phosphorus ratio with another study of RA (rheumatoid arthritis) patients that a decreasing level of calcium-phosphorus ratio [20,44] and researchers have documented alterations of calcium and phosphorous metabolism [20]. Several studies have revealed altered calcium and phosphorus levels in RA patients [20,44-48], but no one has still been attempted for OADs. It is noteworthy to mention here that serum i-PTH level was increased significantly in experimental subjects of combined ($P < 0.001$), females ($P < 0.01$) and males ($P < 0.01$), when compared with that of control subjects (Fig. 5). The reference range of serum i-PTH has been established between 15.0 – 65.0 pg/mL as per Aloia et al. [49]. The present data were observed in increasing trend that an alarming for OAD patients, but within the range between 15 – 65 pg/ml. It was observed that males are more susceptible than female having OADs. In general, i-PTH determined mainly the differentiation of primary hyperparathyroidism from other (non-parathyroid-mediated) forms of hypercalcemia, such as malignancy, sarcoidosis, and thyrotoxicosis [50]. It is well understood that increasing i-PTH leads to osteoclast formation, which has a strong evidence during bone diseases such as OA, RA, osteoporosis etc. [32]. On the other hand, i-PTH has been shown to induce bone resorption in bone marrow cultures [51-52], and in cultures of mixed bone cells from 10 to 15 days old rodents [53-55].

The present study revealed that a close association between lower calcium, phosphorus, calcium-phosphorus ratio and higher i-PTH level in the serum of OAD subjects (Fig. 6), which supported the present data with an evidence for abnormal mineral metabolism [33]. It is quite often that normal homeostatic regulation occurred mainly three target organs viz. intestine, kidney and bone along with the complex integration of two hormones, PTH and vitamin D. Basically low level of serum calcium during abnormal conditions such as bone disorders, kidney diseases, etc. stimulate the secretion of excess PTH [33].

IV. Conclusion

The present results concluded that lowering calcium-phosphorus ratio and higher PTH level may be a suitable diagnostic tool to assay OADs besides radiological and MRI imaging, which is an endeavor to report in the first time. It has already been established that excess dietary intake of calcium and phosphorus as a supplement during OADs lead to lowering calcium-phosphorus ratio, which had adverse health impacts such as bone loss, skeleton deformities, death, etc. [56].

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Conflict of interest

Author has no conflict of interest in the present study.

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