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Biomarkers Of Osteoarthritis In Pakistani Population

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Abstract

Osteoarthritis, the disease mostly caused by the destruction of protective cushions of the bone is diagnosed mainly by X ray radiographs and MRI. Biomarkers like Vitamin D level, Serum Calcium level, Parathyroid hormone level, Erythrocyte Sedimentation Rate, Rheumatoid Factor, Uric Acid level, Bone Alkaline Phosphatase (BAP) level, Hyaluronic Acid (HA), High sensitivity C Reactive Protein (hs-CRP), BMI and platelet count are helpful for the early diagnosis and effective treatment of the disease. The study was performed (total 200 participants) by dividing them into 4 groups, 2 groups are of healthy controls (male and females) and 2 groups are of diseased participants (male and female). Level of all above mentioned biomarkers was assessed in male and female groups by commercially available kits and laboratory tests.Serum Calcium level, Parathyroid hormone level, Erythrocyte Sedimentation Rate, Uric Acid level, Bone Alkaline Phosphatase (BAP) level, Hyaluronic Acid (HA), High sensitivity C Reactive Protein (hs-CRP) are found significantly important (P< 0.0001) biomarker for osteoarthritis. While Vitamin D level, BMI, Rheumatoid Factor and platelet count are not found to be significantly important (P> 0.0001) biomarker for osteoarthritis. To avoid worsening of disease and early diagnosis of the disease these parameters must be measured in patients.

Introduction

Osteoarthritis (OA) is one of the major health problem and its severity increases up to 30% as the age increases (Van Saase et al., 1989 and Spector & Hart, 1992). It is one of the most common musculoskeletal disease which results in the disability of joints mostly in elderly persons (Felson, 2003). Many factors are involved in the onset of OA including destruction of cartilage, modifications in synovial membrane and the development of subchondral bone in the joints (Pelletier & Pelletier, 2007). No. of processes are involved in the onset of OA. Inflammation and obesity (Davis et al., 1990) is one of these factors that triggers the cartilage damage in OA (Sellam and Berenbaum, 2010and Das, 2001).Cytokines notably interleukin-1beta (IL-1β), interleukin-6(IL-6) and tumor necrosis factor-alpha (TNF-α) are thought to be associated with the development and pathogenesis of OA(Kapoor et

al., 2011). Metabolic dysfunctions (Bosello and Zamboni, 2000) and activation of pro-inflammatory mediators are also involved in OA(Vuolteenaho et al., 2009). Studies have shown the prevalence of knee osteoarthritis (KOA) in China, India and Bangladesh. A study conducted in Pakistan has also shown that 28.00% of the urban and 25.00% of the rural population have knee osteoarthritis (KOA) (Iqbal et al., 2011).

The incidence of OA can be measured by multiple ways including intensity of pain and function of the joint, radiographs, magnetic resonance imaging (MRI) and biomarkers. Ultra sound and MRI, the imaging markers, act as potent biomarkers for the evaluation of OA (Eckstein & Wirth, 2010, Eckstein et al., 2012 and Iagnocco et al., 2012). However, these imaging markers especially MRI is limited to use because of its cost (Lotz et al., 2013). Among these methods, detection of the concentration of diseased-specific biomarker in the blood and/or tissue is one of the most prominent and quick method for the diagnosis of any disease. Typically, biomarkers are thought to be some biochemical substances that varies in terms of concentration and/or physiology in diseased condition from that of normal condition. Diseased condition could be precisely and delicately detected by measuring the concentration of diseased-specific biomarkers (Fung et al.,2000). Biomarkers may be fragments of RNA and DNA, biochemical substances or a blend of all these helpful for the diagnosis of certain diseases (Bauer et al., 2006). The concentration and/or physiology of the biomarker may be changed due to metabolic processes at the sites of infection. Detection of biomarkers, either for diagnostic purpose or for drug development, is encouraged by FDA (Gotman& Hackett, 2006). Biomarkers for osteoarthritis have been classified into five categories: (i) diagnostics, (ii) prognostic, (iii) burden of disease, (iv) efficacy of intervention and (v) investigative purpose (Bauer et al., 2006).

Mast cell activation (Buckley et al., 1997),Serum triglycerides, cholesterol level(Dahaghin et al., 2007), body mass index (BMI)(Coggon et al. 2001 and Al-Arfaj, 2003), erythrocyte sedimentation rate (ESR) (Ramirez et al., 2014 andCudejko et al. 2016), rheumatoid factor (RF) (Altman et al., 1986), bone alkaline phosphatases (BAP) level (Hulejová et al., 2003 and Trumble et al. 2008), platelet count, neutrophil count (Balbaloglu et al., 2014), C-reactive protein (CRP) (Tamm et al., 2005,Balbaloglu et al., 2014, Ramirez et al., 2014 and Joshi et al., 2016), vitamin-D level (Mabey and Honsawek, 2015 and Glover et al., 2015) are thought to be the potent and economical biomarkers for osteoarthritis.

As the radiological identification of OA is not economically favorable for the patients so my main object is to find out those potent serum biomarkers which are economically favorable for the patients and helpful in the early diagnosis of OA to enhance the life style of the individuals.

Materials and methods

Selection of patients

Patients have been selected from different regions of Punjab the province of Pakistan having definite osteoarthritis identified by the professors of orthopaedic wards of tertiary and secondary hospitals including Mayo hospital Lahore (The division of province Punjab), Allied hospital Faisalabad (The division of province Punjab), Allama Iqbal memorial hospital Attawa (Gujranwala the division of province Punjab) and their pain, stiffness and physical functions have been accessed by “The Western Ontario and McMaster Universities Osteoarthritis Index” (WOMAC). Patients are then divided into four different groups.

Study protocol

This study was a cross-sectional study, using the data from the teaching and non-teaching hospitals of the Punjab the province of Pakistan from March 2016 to March 2017. Total 200 participants are taken, and they are divided into four groups in such a way that two groups are of male having 50 participants in each group one is designated as group I that is of effected persons and group II that is of controlled persons. Other two groups are of females each containing 50 persons designated as group III of effected females and group IV having controlled females. All 200 participants having age more than 40 years. The participants were then passed through the radiographic examination of the knees to confirm osteoarthritis. Body Mass Index (BMI) was calculated for all the 200 participants.

Collection of blood

Blood is collected from 200 participants in vacutainers. Serum was obtained at 2500 rpm. The serum samples were then stored at -40 °C in biochemistry lab of GCUF. The collected serum was then processed for the tests given below

1	Vitamin D Level	
2	Serum Calcium Level	
3	Parathyroid hormone Level	(PTH level)
4	Erythrocyte sedimentation rate (ESR)	
5	Rheumatoid factor	(RF)
6	Uric acid	
7	Bone Alkaline Phosphatases	(BAP)
8	Hyaluronic acid (HA)	
9	High sensitivity C receptor protein	(hs-CRP)
10	Platelet count	

Procedure to biochemical tests

A blend of different procedures is performed for the detection of molecules. Serum obtained from blood by centrifugation at 2500 rpm for 15 minutes is subjected to a). coagulation method b). calorimetric method c). immunological tests d) redox reactions e). haematological tests for the detection of rheumatoid factor, uric acid, bone alkaline phosphatase, vitamin D level, hyaluronic acid, para thyroid hormone level, high sensitive C reactive protein, calcium level, erythrocyte sedimentation rate and platelet count. Coagulation method, Calorimetric method and immunological methods are performed by commercially available kits. The whole procedure applied for the detection of these biomarkers has been summarised in figure 1.

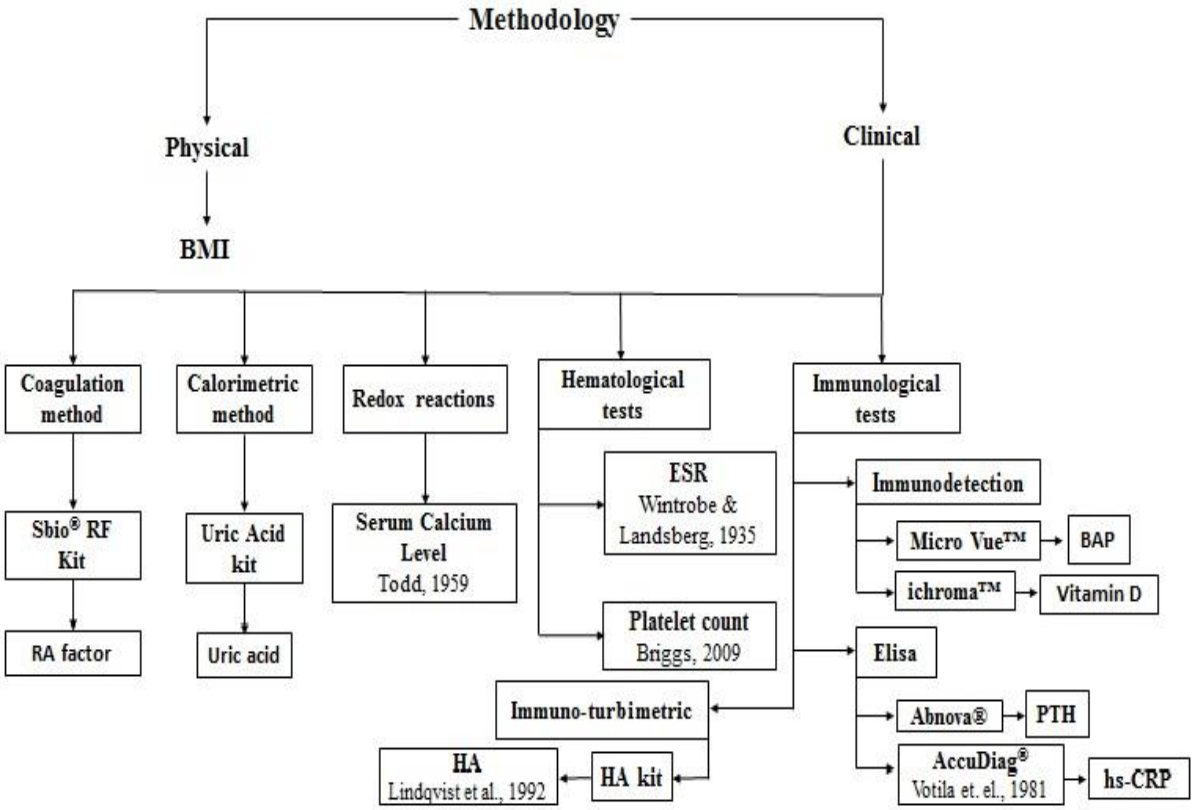

Figure 1: Schematic diagram of methods applied for the detection of biomarkers.

Results

Participant Characteristics

Clinical features of participants have been summarised in Table 1. Participants have mean age of 50±10 years. BMI in male control group is 25.18±3.42 kg/m^2 and in male diseased group it is 25.35±3.28 kg/m^2. Infemales both in control and diseased groups it is 28.05±4.35 kg/m^{2}and 27.4±4.2kg/m^2.Systolic BP in all four groups is 120-125mmHg, 140-150mmHg, 100-115 mmHg and 130-140 mmHg respectively. Diastolic BP is 85-95 mmHg, 80-100 mmHg, 80-90 mmHg and 90-100mmHg respectively. Fasting and random glucose level of all the four groups is in normal range and is summarized in the following table. Table 1 also indicates that all the participants are normal except the patient's group have only osteoarthritis.

Table 1: Clinical features of study participants.

Parameters	Male participants			Female participants		
	Control males	Diseased males	P-value	Control females	Diseased females	P-value
Numbers	50	50	-	50	50	-
Age (years)	40-60	40-60	--------	40-60	40-60	--------
BMI (kg/m^2)	25.35 ± 2.24	25.182 ± 2.38	> 0.05	26.52 ± 2.02	27.67 ± 2.56	< 0.05
Systolic BP (mmHg)	120-125	140-150	< 0.001	100-115	130-140	< 0.001

Diastolic BP (mmHg)	80-90	80-85	< 0.001	70-80	80-90	< 0.001
Fasting Glucose Level (mg/dl)	85-95	80-100	> 0.01	80-90	90-100	< 0.001
Random Glucose Level (mg/dl)	110-120	100-120	< 0.001	90-120	110-120	< 0.001
Vitamin D level (ng/ml)	32.6 ± 3.84	32.33 ± 10.56	> 0.05	27.85 ± 1.52	27.38 ±1.34	> 0.05
Serum Calcium Level (mg/dl)	9 ± 0.6	6.758 ± 0.55	< 0.0001	8.534 ± 0.57	6.088 ± 0.51	< 0.0001
Parathyroid hormone level (pg/ml)	33.66 ± 10.14	58.36 ± 4.70	< 0.0001	37.12 ± 8.59	55.4±4.99	< 0.0001
Erythrocyte sedimentation rate (mm/Hr)	17.88 ± 1.87	37.74 ± 20.65	< 0.0001	22 ± 3.69	41.64 ± 16.33	< 0.0001
Uric acid (mg/dl)	4.978 ± 1.15	7.86 ± 0.47	< 0.0001	4.016 ± 1.17	6.592 ± 1.21	< 0.0001
Bone Alkaline Phosphatases (µg/L)	10.4 ± 5.5	28.5 ± 4.99	< 0.0001	15.56 ± 3.77	32.28 ± 4.39	< 0.0001
Hyaluronic acid (ng/ml)	36.812 ± 0.89	43.666 ± 2.93	< 0.0001	37.28 ± 1.24	44.58 ± 3.28	< 0.0001
High sensitivity C reactive protein (mg/L)	1.378 ± 0.47	3.682 ± 0.47	< 0.0001	1.736 ± 0.4	3.816 ± 0.52	< 0.0001
Platelet count (per mm^3)	188100 ± 26557	186000 ± 30538	> 0.05	183800 ± 32598	180320 ± 28669	> 0.05
Rheumatoid Factor	0 % in participants	10 % in participants	> 0.05*	0 % in participants	8 % in participants	> 0.05*

1. * t-Test: Paired Two Sample for Means

BMI and Osteoarthritis

Mean BMI was 25.18 ±2.38 Kg/m² (Fig. 2) in OA male groups and 25.35 ±2.24 Kg/m² (Fig. 2) in the male control group (Table 1). Statistically both the groups have no high significant difference (P> 0.05). Similarly, mean BMI was 27.72 ± 2.56 Kg/m² (Fig. 2) in OA female groups and 26.55 ± 2.02 Kg/m² (Fig. 2) in female control group (Table 1) which is significant (P< 0.05) indicating that BMI has significant role in the onset of OA in females.

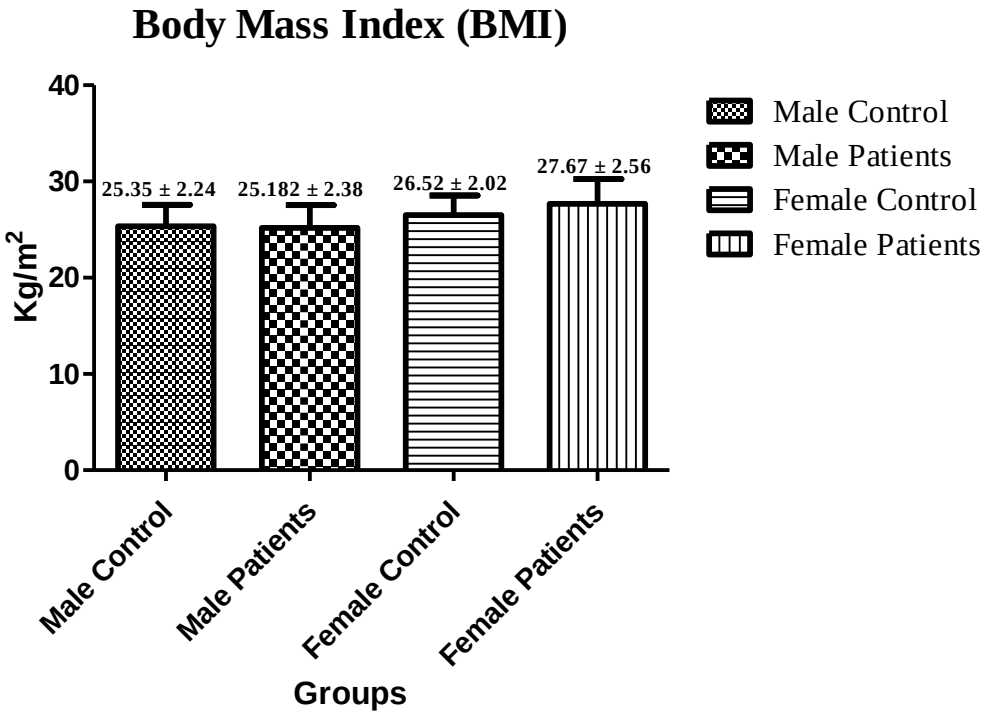

Fig. 2: Mean values of BMI in groups under study

2. Vitamin D and Osteoarthritis

Mean Vitamin D level was 32.33 ± 10.56 ng/ml (Fig. 3) in OA male groups and 32.6 ± 3.84 ng/ml (Fig. 3) in the male control group (Table 1). Statistically both the groups have no significant difference (P> 0.05). Similarly, mean Vitamin D level was 27.38 ±1.34 ng/ml(Fig. 3) in OA female groups and 27.85 ± 1.52 ng/ml (Fig. 3) in female control group (Table 1) which is also has no significant difference (P> 0.05) indicating that Vitamin D has no significant role in the onset of OA in females.

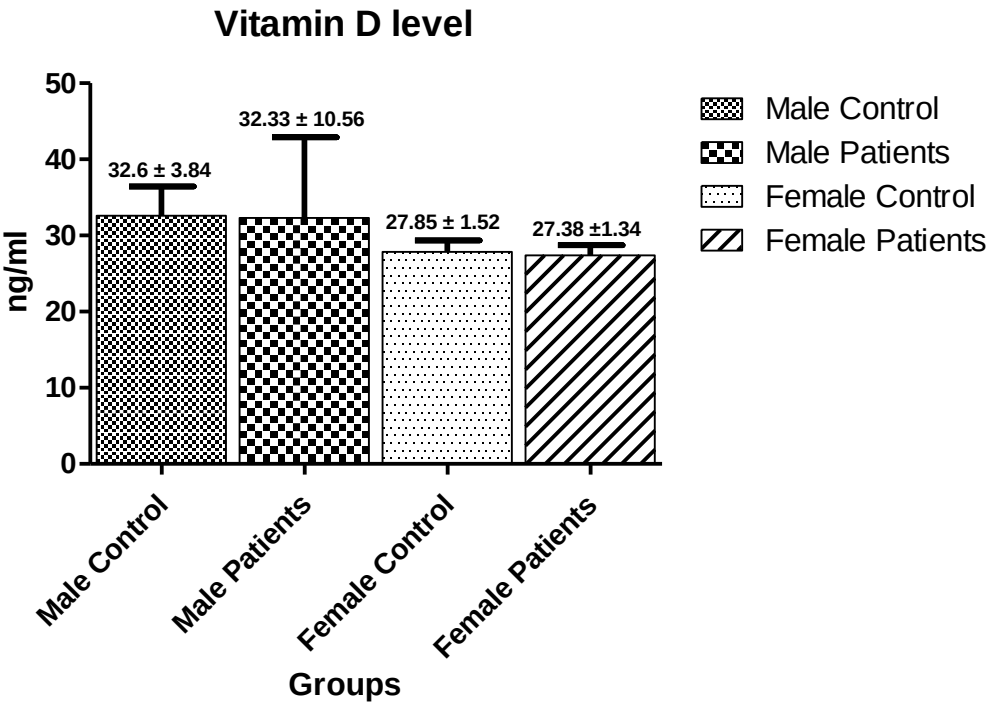

Fig. 3: Mean values of vitamin D level in study groups

3. Serum Calcium Level and Osteoarthritis

Mean Calcium level was 6.758 ± 0.55 mg/dl (Fig.4) in OA male groups and 9 ± 0.6 mg/dl(Fig.4) in the male control group (Table 1). Statistically both the groups have high significant difference (P< 0.0001)indicating that serum Calcium has significant role in the onset of OA in males. Similarly, mean Vitamin D level was 6.088 ± 0.51 mg/dl(Fig.4)in OA female groups and 8.534 ± 0.57 mg/dl (Fig.4) in female control group (Table 1) which is also highly significantly difference (P< 0.0001) indicating that serum calcium has significant role in the onset of OA in females.

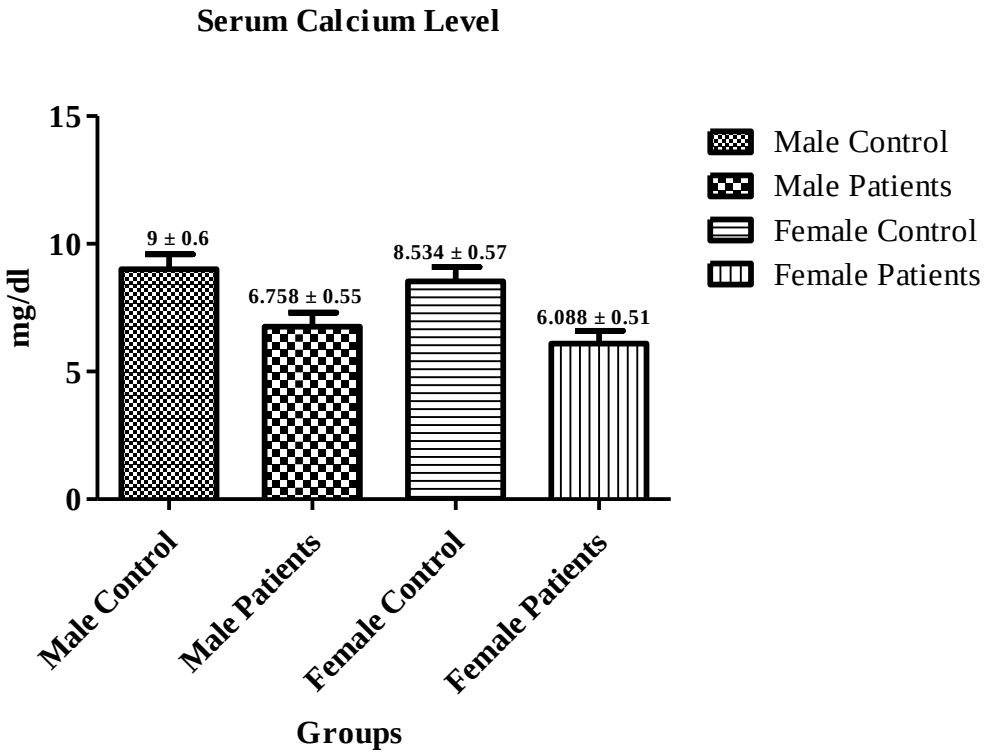

Fig. 4: Mean values of Serum Calcium level in study groups

4. Parathyroid Hormone and osteoarthritis

Mean PTH level was 58.36 ± 4.70 pg/ml (Fig.5) in OA male groups and 33.66 ± 10.14 pg/ml(Fig.5) in the male control group (Table 1). Statistically both the groups have high significant difference (P< 0.0001) indicating that PTH has significant role in the onset of OA in males. Similarly, mean PTH level was 55.4±4.99 pg/ml (Fig.5) in OA female groups and 37.12 ± 8.59 pg/ml(Fig.5) in female control group (Table 1) which is also highly significantly difference (P< 0.0001) indicating that PTH has significant role in the onset of OA in females.

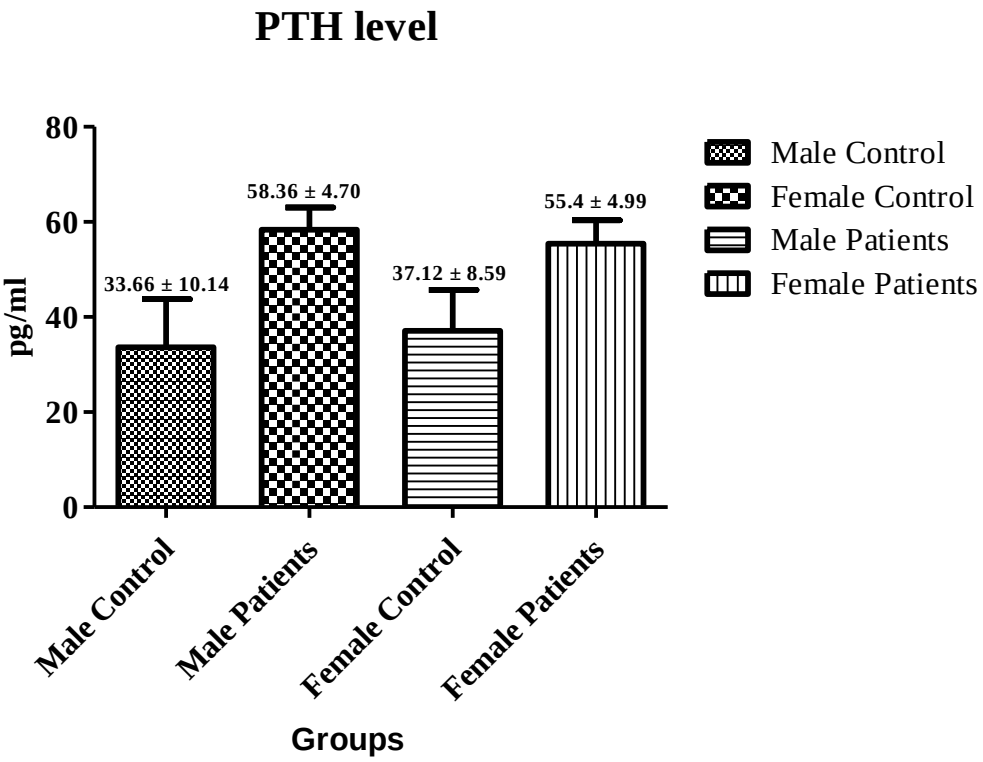

Fig. 5: Mean values of PTH level in study groups

5. Erythrocyte Sedimentation Rate and Osteoarthritis

Mean ESR was 37.74 ± 20.65 mm/Hr (Fig.6) in OA male groups and 17.88 ± 1.87 mm/Hr (Fig.6) in the male control group (Table 1). Statistically both the groups have high significant difference (P< 0.0001) indicating that ESR has significant role in the onset of OA in males. Similarly, mean ESR was 41.64 ± 16.33 mm/Hr (Fig.6)in OA female groups and 22 ± 3.69 mm/Hr(Fig.6) in female control group (Table 1) which is also highly significantly difference (P< 0.0001) indicating that ESR has significant role in the onset of OA in females.

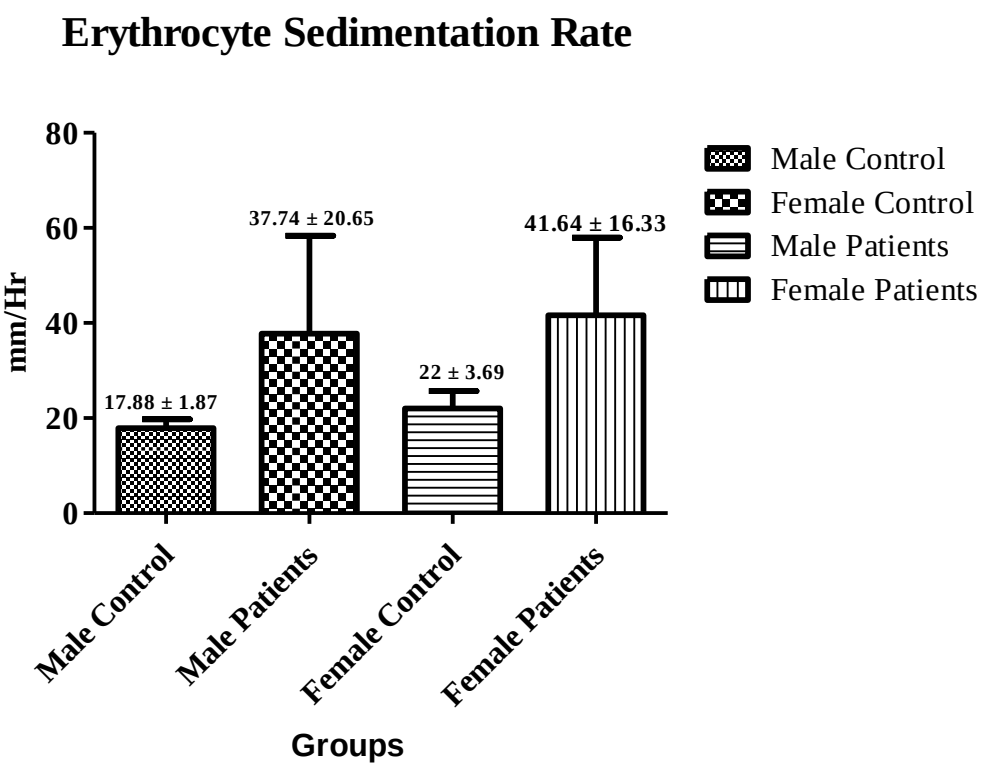

Fig. 6: Mean values of ESR in study groups

6. Uric acid level and Osteoarthritis

Mean Uric Acid Level was 7.86 ± 0.47 mg/dl (Fig.7) in OA male groups and 4.978 ± 1.15 mg/dl (Fig.7) in the male control group (Table 1). Statistically both the groups have high significant difference (P< 0.0001) indicating that Uric Acid has significant role in the onset of OA in males. Similarly mean Uric Acid level was 6.592 ± 1.21 mg/dl (Fig.7) in OA female groups and 4.016 ± 1.17 mg/dl (Fig.7) in female control group (Table 1) which is also highly significantly difference (P< 0.0001) indicating that Uric acid has significant role in the onset of OA in females.

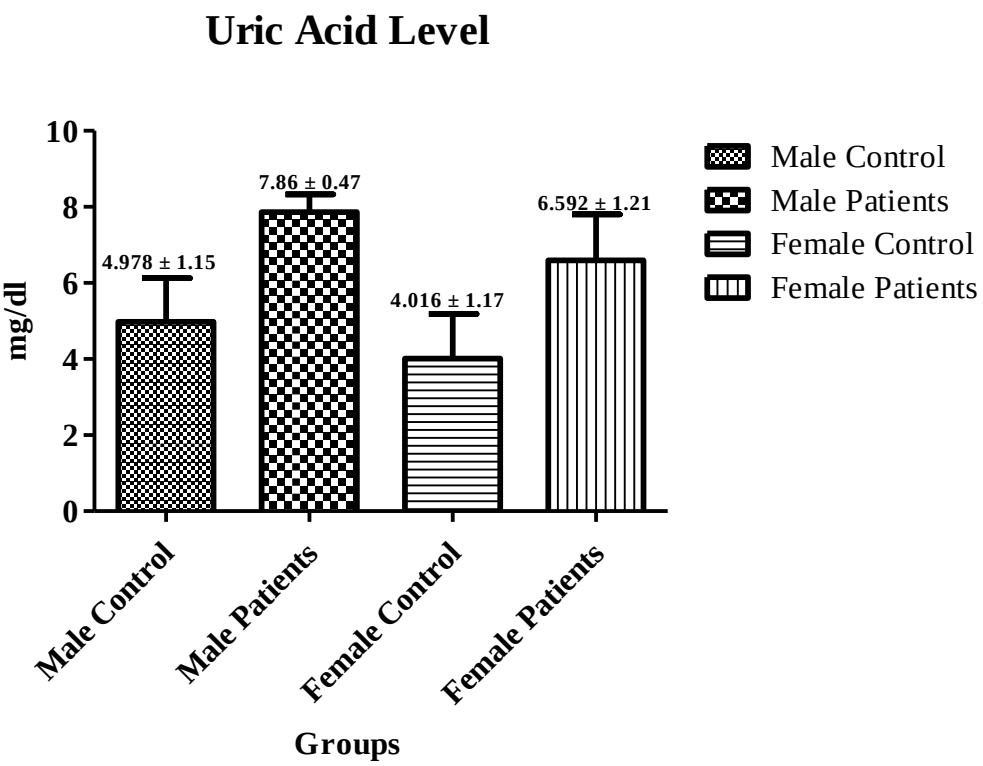

Fig. 7: Mean values of Uric Acid Level in study groups.

7. Bone Alkaline Phosphatases and Osteoarthritis

Mean BAP level was 28.5 ± 4.99 µg/L (Fig. 8) in OA male groups and 10.4 ± 5.5 µg/L (Fig. 8) in the male control group (Table 1). Statistically both the groups have high significant difference (P< 0.0001) indicating that BAP has significant role in the onset of OA in males. Similarly, mean BAP level was 28.5 ± 4.99 µg/L (Fig. 8)in OA female groups and 10.4 ± 5.5 µg/L (Fig. 8) in female control group (Table 1) which is also highly significantly difference (P< 0.0001) indicating that BAP level has significant role in the onset of OA in females.

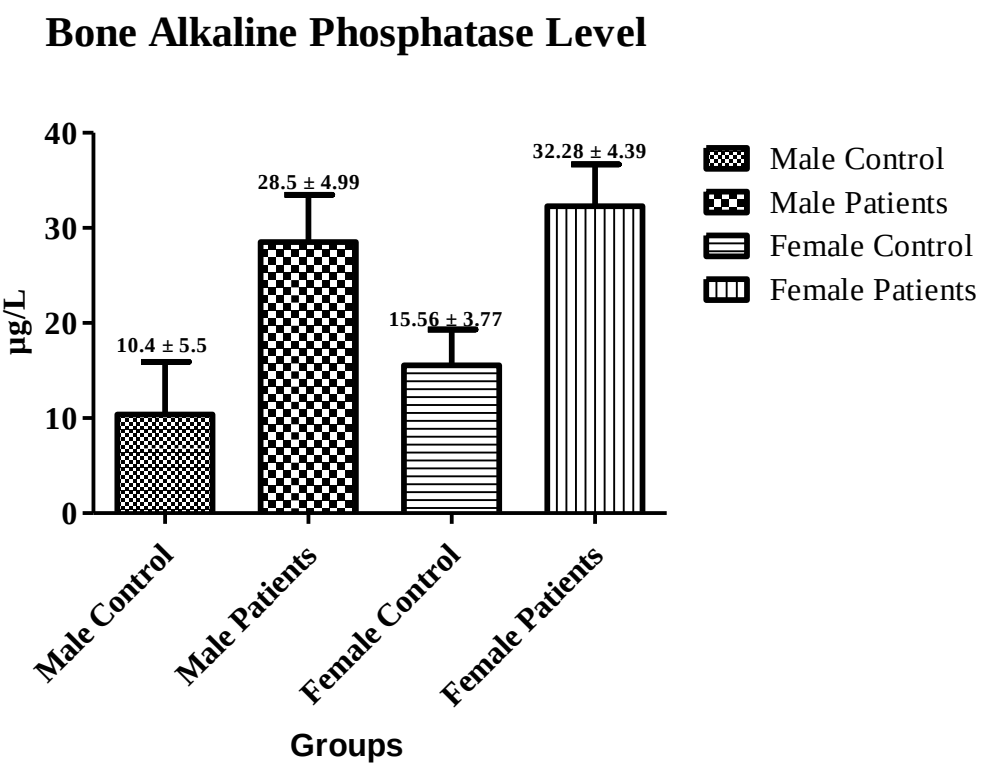

Fig. 8: Mean values of BAP Level in study groups.

8. Hyaluronic acid and Osteoarthritis

Mean HA level was 43.666 ± 2.93 ng/ml (Fig. 9) in OA male groups and 36.812 ± 0.89 ng/ml(Fig. 9)in the male control group (Table 1). Statistically both the groups have high significant difference (P< 0.0001) indicating that HA has significant role in the onset of OA in males. Similarly, mean HA level was 44.58 ± 3.28 ng/ml (Fig. 9)in OA female groups and 37.28 ± 1.24 ng/ml(Fig. 9)in female control group (Table 1) which is also highly significantly difference (P< 0.0001) indicating that HA level has significant role in the onset of OA in females.

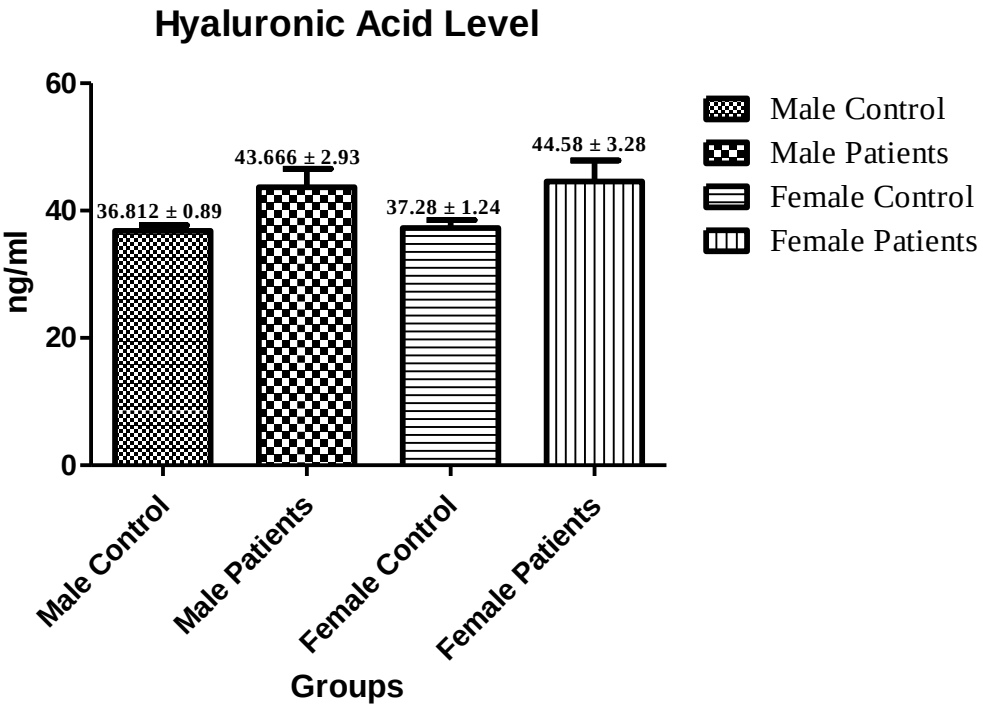

Fig. 9: Mean values of HA Level in study groups.

9. High sensitivity C receptor protein and osteoarthritis

Mean hs-CRP level was 3.682 ± 0.47 mg/L (Fig. 10) in OA male groups and 1.378 ± 0.47 mg/L (Fig. 10) in the male control group (Table 1). Statistically both the groups have high significant difference (P< 0.0001) indicating that hs-CRP has significant role in the onset of OA in males. Similarly, mean hs-CRP level was 3.816 ± 0.52 mg/L (Fig. 10)in OA female groups and 1.736 ± 0.4 mg/L (Fig. 10) in female control group (Table 1) which is also highly significantly difference (P< 0.0001) indicating that hs-CRP level has significant role in the onset of OA in females.

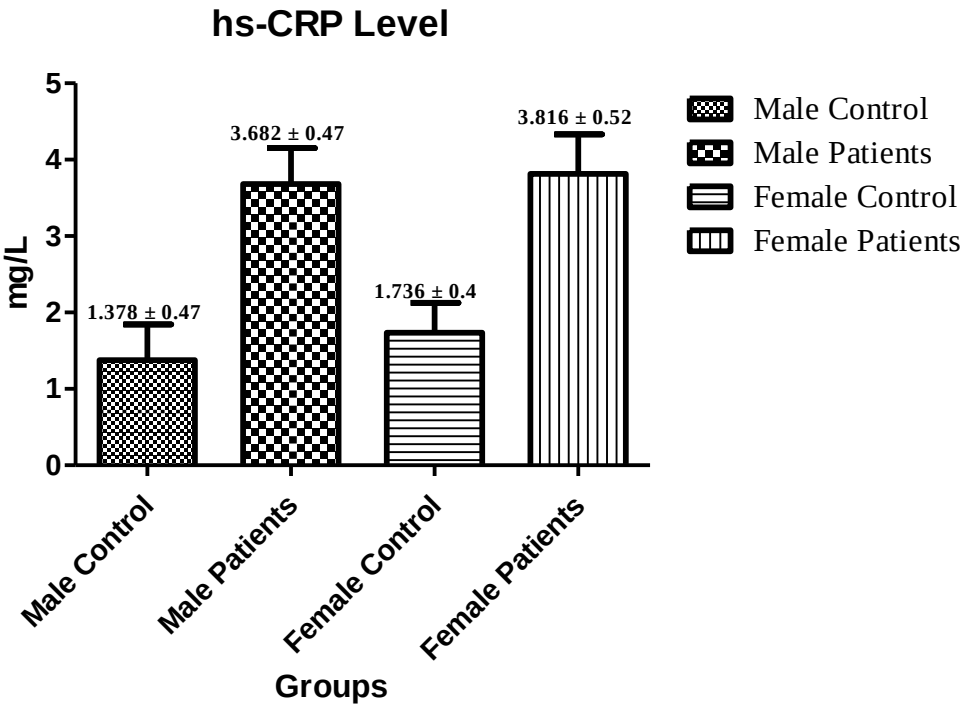

Fig. 10: Mean values of hs-CRP Level in study groups

10. Platelet count and Osteoarthritis

Mean platelet count was 186000 ± 30538 per mm^3(Fig. 11)in OA male groups and 188100 ± 26557 per mm^3(Fig. 11) in the male control group (Table 1). Statistically both the groups have no significant difference (P> 0.05) indicating that platelet count has no significant role in the onset of OA in males. Similarly, mean platelet count level was 180320 ± 28669 per mm^3(Fig. 11)in OA female groups and 183800 ± 32598 per mm^3(Fig. 11) in female control group (Table 1) which is also not significantly difference (P> 0.05) indicating that platelet count level has no significant role in the onset of OA in females.

11. Rheumatoid Factor and Osteoarthritis

According to statistical analysis the P value of male groups is > 0.05*(Table 1) which is greater than level of confident 0.05 so the means of both groups are not significantly different from each other. Similar type of result was seen in female groups where P value is >0.05*(Table 1) showing that no significant difference is there between them. But Rh Factor in male is more common 10% (Figure 12) than in females 8% (Figure 12). So from our results we can conclude that there is no effect of Rheumatoid Factor on osteoarthritis.

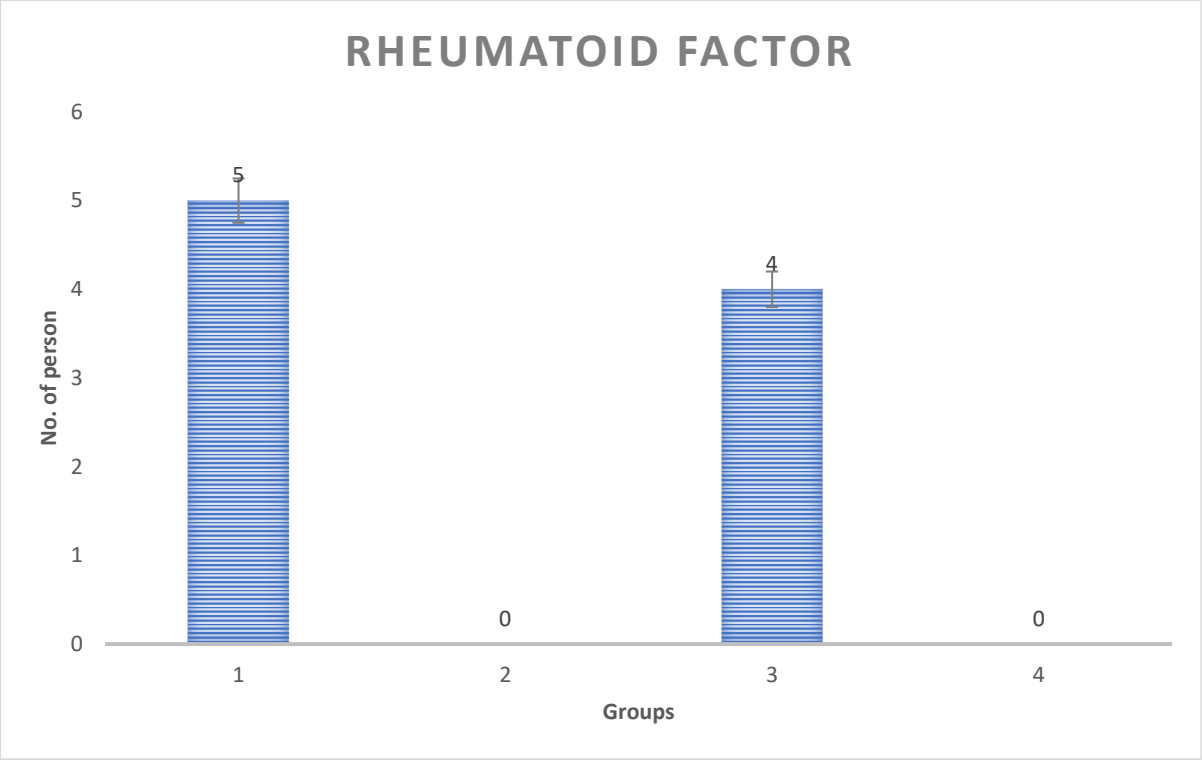

Figure 12: Graph showing no of effected persons due to Rh Factor in all four groups

Discussion

A degenerative disorder of joints leading to the destruction of bone cartilage as well as formation of new bones at the joints is osteoarthritis (OA). Onset of the disease is more in older individuals specially after the age of 40 years. Severity of disease is more in women then in men (Lawrence et al., 1998).

Standards of life has been compromised by the osteoarthritis. Exact cause of the disease is also unknown. Trauma, inflammation, fractures and immobilization of joints are some potent risk factors which may lead to the elevation or reduction of potent biomarkers. Various studies have been performed to find out the diagnostic biomarker, so the disease can be diagnosed earlier and can be treated earlier to improve the standards of life.

During this study 100 patients of OA (male and female) are compared with same numbers of sex matched controls having same age. Higher BMI results in the obesity which leads to the knee osteoarthritis (Niu et al., 2009) by destroying cartilage (Hamblen and Simpson, 2009). Onset and progression of OA is highly associated with increased body mass index (BMI) (Reijmanet al., 2007).BMI is significant ($p = 0.05$) in onset of OA (Singer et al., 2018). In our study BMI is also found significant ($P< 0.05$) in females but in males it is found to be non-significant ($P > 0.05$).

Serum Calcium level and PTH levels are thought to be the most important biomarkers. In my experiments that have been performed $P< 0.05$ which means that both are significant in the development of OA. Because of its significant difference OA may develop due to elevated PTH level. The result is supported by the work of Orth et al., 2014. They explained the mechanism that how

increased PTH causes the decrease in ca level and eventually causes the degradation of cartilages at the cap of the bones.

Of all markers of severity and extent of OA assessed, intensity of pain was most strongly associated with low level rises in the mean serum hs-CRP levels. in this group of patients with knee OA were associated with serum hs-CRP levels. There is growing evidence that systemic markers of inflammation are associated with severity or clinical course of OA. Serum CRP, probably the most widely used clinical marker of systemic inflammation, has been shown to correlate well with CRP in synovial fluid in patients with OA. (Kumon et al., 1999). Their information about hs-CRP also supports my experiment because hs-crp is found to be highly significant in OA.

Elevated Hyaluronic acid (HA) in the serum also an indicator of potent biomarker of osteoarthritis. My results of experiments were supported by the work of Sharif et al. in 1995. Other biomarkers like platelet count, Rheumatoid Factor are not significant in the progression and development of osteoarthritis. ESR the other marker is found to be significant, but this may be due to the infection produced at the site of arthritis. Highly decrease in P value causes the high risk of OA in individuals. The result was supported by Denoble et al. in 2011 when they were explaining the activation of certain biochemicals in the body due to uric acid.

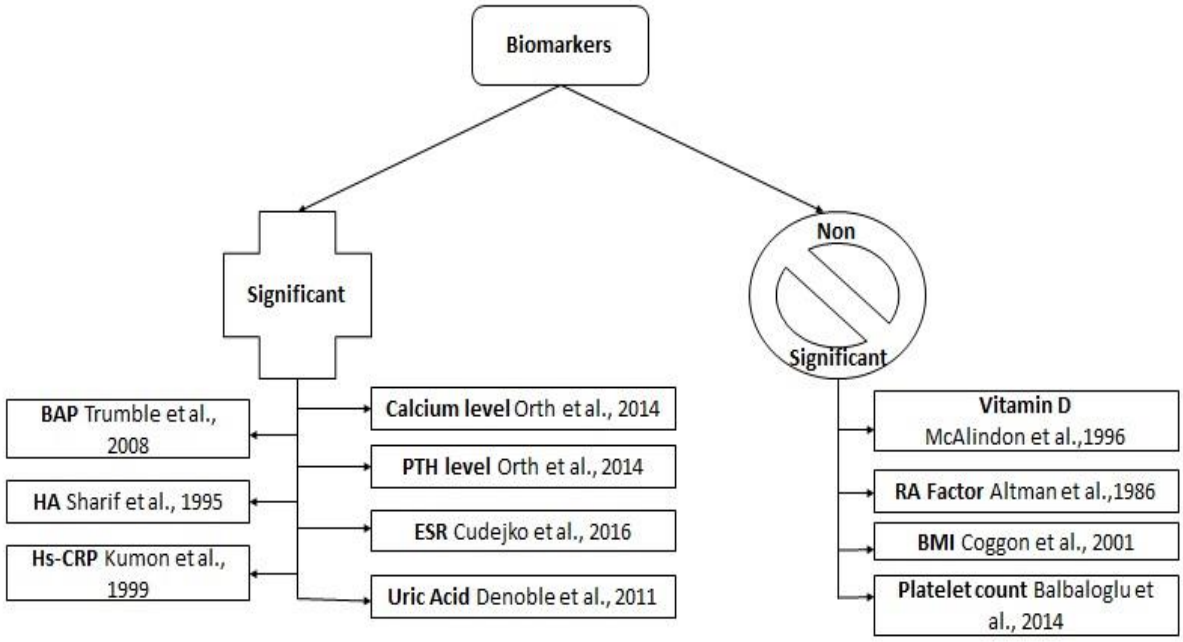

Figure 13: Schematic diagram of biomarkers significant and non-significant to OA.

Conclusion

Bones diseases especially OA are most common in elderly patients. With the onset of disease, Vitamin D level, Serum Calcium level, Parathyroid hormone level, Erythrocyte Sedimentation Rate, Rheumatoid Factor, Uric Acid level, Bone Alkaline Phosphatase (BAP) level, Hyaluronic Acid (HA), High sensitivity C Reactive Protein (hs-CRP), BMI and platelet count varies from that of normal values both in male and female. These markers may helpful for the clinical diagnosis of OA. More research

is on the way to find out the exact mechanism by which these markers shows there mark able effect during OA.

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