DISCUSSION

Osteoarthritis is a chronic, painful, age-related disorder that has a substantial impact on quality of life (Woolf et al., 2003). It is a heterogeneous group of disorders characterized pathologically by focal areas of loss of cartilage in synovial joints that is associated with varying degrees of osteophyte formation, subchondral bone change which grip the whole joint, i.e. articular cartilage, subchondral bone alterations and joint-lining synovial membrane inflammation (Bonnet et al., 2005; Ashraf et al., 2014). Chondrocytes are the unique cell type of adult human articular cartilage capable to maintain extracellular matrix components integrity and turnover (Martin et al., 2002). In osteoarthritis, due to abnormal environmental insults, chondrocytes produce a wide range of inflammatory mediators leading cartilage loss of entire joint structures includes hands, knee, ankle and long bones (Heinegard et al., 2011). Nowadays, it is also known as low grade chronic inflammatory condition. Disease progression is usually slow but can ultimately lead to joint failure with pain and disability.

Worldwide estimates are that 9.6% of men and 18.0% of women aged ≥60 years have symptomatic osteoarthritis whereas in India it ranges from 14-47% (Woolf et al., 2003; Salve et al., 2010; Ganvir et al., 2013). The prevalence of osteoarthritis increases indefinitely with age because the condition is not reversible. Before the age of 45, more men than women have osteoarthritis but after age 45, it is more common in women (Davis et al., 1988; Akinpelu et al., 2009). Pain is the initial and the core clinical feature in both sex followed by impaired joint mobility, reduction of muscle strength and loss of joint function (Felson, 2006; Lane et al., 2007) while the other symptoms and signs characteristic of osteoarthritis in the most frequently affected joints are heat, swelling, stiffness and bony enlargement. This disease is very common
in postmenopausal women as compared to premenopausal women. The lower estrogen level may be the important regulator of osteoarthritis. Estrogen can influence chondrocytes function on multiple levels by interacting with cellular growth factors, adhesion molecule and cytokines (Gokhale et al., 2004). The underlying biochemical mechanism of osteoarthritis is the biomechanical stress which causes various structural and biochemical changes that result in the production of proinflammatory cytokines and activate an inflammatory chemical reaction cascade in articular tissues. Chondrocytes of OA patients actively produce prostaglandins, TNF-α, IL-1β, IL-6 and IL-8 (Pelletier et al., 2001). Recent studies demonstrate inflammatory changes in the synovial membrane of OA patients, including synovial hypertrophy and infiltration of the underlying tissue by various inflammatory cells like monocytes, neutrophils and leukocytes (Smith et al., 1997; Haywood et al., 2003). Leukocytes play a pivotal role in inflammation because of their antimicrobial, secretory, and phagocytic activities (Ulbrich et al., 2003). A primary feature of the inflammatory response is the recruitment of leukocytes from the vessel wall into inflamed tissue and their interaction with other cell types, such as macrophages and fibroblasts (Carlos et al., 1994; Ulbrich et al., 2003). Intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) play important roles in endothelial-leukocyte interactions (Ulbrich et al., 2003). Their regulated expression affects the binding and transmigration of leukocytes during inflammation (Carlos et al., 1994). Risk factors for osteoarthritis in general are aging, genetic factor, obesity, diabetes, dyslipidemia and hypertension. Many studies showed that diabetes is responsible for the onset of osteoarthritis in both sexes.

Diabetes is a chronic metabolic disorder characterized by hyperglycemia and glycosuria which is due to deficiency of insulin or β-cell dysfunction (Smith et al.,
Diabetes is classified into two major categories- Type I Diabetes Mellitus and Type 2 Diabetes Mellitus. Type 1 diabetes or juvenile-onset diabetes, results from cellular mediated autoimmune destruction of β- cells of the pancreas, resulting in the production of insufficient amount of insulin. Type 2 diabetes or adult onset diabetes occurs due to insulin resistance and absolute or relative deficiency of insulin (Atkinson et al., 1994; DeFronzo et al., 1992). Type 2 diabetes is reported to be associated with osteoarthritis. Hyperglycemia and osteoarthritis interact at both local and systemic level. The increased concentration of glucose in cartilage matrix induces the oxidative stress leads to the production of ROS and overproduction of proinflammatory cytokines which leads to matrix stiffness, subchondral bone destruction and chondrocytes dysfunction (Berenbaum, 2011; Zhuo et al., 2012). Hyperglycemia, hyperinsulinemia or insulin resistance may be responsible for the elevation of adhesion molecules (Morigi et al., 1998; Chen et al., 1999; Matsumoto et al., 2000). Increase in sVCAM-1 may partly explain the predisposition of diabetic osteoarthritis in postmenopausal women. Therefore we framed our study to know whether the release of soluble VCAM-1 in diabetic and even in non-diabetic postmenopausal women subjects helpful in early diagnosis of osteoarthritis.

The present study has been undertaken by taking diabetic (n=150) and non-diabetic (n=150) postmenopausal female subjects of age group 45-75 years with and without osteoarthritis. Various biochemical parameters like sugar, lipid profile, uric acid, C-reactive protein along with osteoarthritis biomarker (sVCAM-1) have been estimated.

In our study, Table No.1-3, Pie-chart 1-3 showing the distribution of subjects selected for the study. We found that the prevalence of osteoarthritis increases with the age though osteoarthritis affect peoples of all age group but elderly persons (60 years) have a high incidence of osteoarthritis. In addition, its prevalence is more common in
elderly women who are in postmenopausal phase as compared to elderly male subjects. The lifestyle also affects the onset of osteoarthritis. It is strongly associated with higher body weight or obesity. Our study showed high BMI in both diabetic and non-diabetic as compared to age-matched healthy control postmenopausal females (Table No. 4, Graph No.4). This is same as quoted by Felson et al., 1988; Spector et al., 1994; Sandmark et al., 1999; Aoyagi et al., 2002; Reijman et al 2007; Sudo et al., 2008; Sowers et al 2009; AL-Rubia et al., 2011; Kidwai et al., 2016; Liu et al., 2016; Tondare et al., 2017). It may be due to the pressure exerted on the articular cartilage increases, which accelerates degeneration. The relationship between BMI and osteoarthritis could also be modified by trauma of the joint, particularly the knee joint. Englund et al., 2004 reported that patients who had undergone total meniscectomy with obesity (BMI >30 kg/m$^2$) had a greater likelihood of knee radiographic osteoarthritis than those with a BMI <25 kg/m$^2$. Leptin, a small polypeptide that regulates food intake and energy expenditure at the hypothalamic level, may provide the metabolic link between obesity and osteoarthritis. Plasma levels of leptin strongly correlate with fat mass, and levels fall after weight loss (Friedman et al., 1998). Recent studies detected functional leptin receptors on human adult articular chondrocytes (Figenschau et al., 2001; Dumond et al., 2003). Leptin may also play a part in the development of osteoarthritis through changes in the bony matrix (Ducy et al., 2000; Aspden et al., 2001). Hence, obesity and diabetes are major risk factors for the development of osteoarthritis. Physical activity is a key component of diabetes mellitus and osteoarthritis management. The mechanism of this comorbidity remain uncertain; these co-occurring diseases have been associated with a significant reduction in quality of life and increased risk for other severe complications.
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Uncontrolled hypertension is another factor for predisposing osteoarthritis. The mechanism of hypertension induced osteoarthritis begins with narrowing of blood vessels, reducing blood flow especially in small subchondral vessels at the ends of long bones by the development of microemboli in subchondral vessels which leads to subchondral ischemia. The ischemia might compromise nutrient and gas exchange between articular cartilage and bone, initiating degradative changes in cartilage (Imhof et al., 2000; Findlay et al., 2007). Another possibility is that apoptosis of osteocytes in the ischemic regions of subchondral bone might trigger osteoclastic resorption, reducing bony support for overlying cartilage (Berger et al., 2003). Though in our study we did not found very high or uncontrolled hypertension in osteoarthritis diabetic and non-diabetic postmenopausal female subjects but the rise is significant as compared to control postmenopausal women (Table No: 4, Graph No.4). This may be because these patients were on antihypertensive medicine. This is supported by Hart et al., 1995; Sowers et al., 2009; Yoshimura et al., 2012; Wen et al., 2013; Liu et al., 2016.

The overall status of biochemical parameters, inflammatory parameter and adhesion molecule in healthy, diabetic and non-diabetic postmenopausal women with and without radiological findings of osteoarthritis in two age groups (1) 45-60 years and (2) 61-75 years is shown in Table No. 5-11, Graph No. 5-11.

The postmenopausal condition in women starts from the age of 45 onwards and associated with deficiency of estrogen hormone. In our study, we observed in non-diabetic postmenopausal women with complain of knee pain, upon radiological investigation no change in X-ray was reported. Pain may be associated with elevated inflammation primarily under conditions of chronic stress. When faced with additional stress, the stress, aggravation, and life impact of pain may cause systemic
inflammation or may exacerbate inflammation already caused by underlying physical pathology. The biochemical parameters like FBS, HbA1c, lipid profile, uric acid were within the normal as compared to control group (p=NS). The status of sVCAM-1 was also found non-significant while the level of CRP was found significantly increased but within normal range (p<0.05) and estrogen was found significantly decreased (p<0.05) (Table No. 12, Graph No. 12a-12e). The increased level of CRP in knee pain in our study is inconsistent with the findings of Saxne et al., 2003 while the decrease level of estrogen in knee pain is in agreement with the study of Chlebowski et al., 2013. Thus, in non-diabetic postmenopausal women, joint pain may be due to deficiency of estrogen hormone (Gao et al., 2010). Jochems et al., 2005 and Hoegh-Andersen et al., 2004 reported that deficiency of estrogen influence on joint pain and inflammatory markers. Estrogen is cardioprotective and anti-inflammatory hormone (Straub et al., 2007). Cartilage is a sex hormone-sensitive tissue. Estrogen and compounds acting through the estrogen receptor exert a chondroprotective effect. It modulates the osteoblast activity, inhibiting calcium removal interference, and reducing osteoclast formation (Nevitt et al., 1996; Martin-Millan et al., 2013; Gambacciani et al., 2014). Cell-cell interaction is a key event during cartilage formation and growth and is mediated by adhesion molecules such as N-cadherin, neural cell adhesion molecule, intercellular adhesion molecule and vascular cell adhesion molecule (Fang et al., 1999). Studies involving the stage-specific expression of these proteins during chondrogenesis have shown that these proteins are developmentally regulated and act at specific stages (Tavella et al., 1994). For example, N-cadherin initiates cell condensation, and NCAM stabilizes the process and changing estrogen levels during growth and maturation can affect their activity (Fang et al., 1999). One effect of low estrogen levels can be the reduction in estrogen
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binding with estrogen receptors. Results of in vitro studies with mice and rats indicated that both the number of estrogen receptors as well as estrogen binding decreased with age (Pinus et al., 1993). The decrease estrogen level in our study did not affect the lipid profile and status of sugar level. The role of inflammation in predisposing any kind of pain can be detected by CRP level (Sturmer et al., 2005). CRP is an acute phase protein that was discovered over 70 years ago to be a blood protein that binds to the C-polysaccharide of pneumococci (Gabay et al., 1999). Any inflammatory stimuli in the tissue will lead to increase in the IL-1, IL-6 and TNF-α which cause increase in CRP and this CRP will increase the tissue factor and adhesion molecule. CRP can also induce the endothelial cell to produce VCAM-1 (Yeh, 2004). In our study since we did not get any change in sVCAM-1 hence this increase CRP level explains the presence of tissue inflammation may be due to external injury. In the same age group (45-60 years) diabetic postmenopausal women with knee pain, hyperglycemia and dyslipidemia are present (Table No.13, Graph No.13a,b) which is consistent with the study of Ajam et al., 2012; Rouen et al., 2015; Chamba et al., 2016. The changes in total cholesterol, LDL-C, TG, VLDL-C and HDL-C level may be due to hormonal changes occurring during menopause or due to insulin resistance. The decline in HDL-C level can be attributed to the increase in cholesterol ester transfer protein (CETP) activity and increased activity of liver lipase. The opposite effect of those enzymes will lead to increased level of LDL-C (Coni et al., 2000). The increase in TG level can be attributed to decreased insulin level or activity in diabetic postmenopausal women which lead to a subsequent decrease in lipoprotein lipase activity with a concomitant increase in VLDL-C levels (Parks et al., 2000). The level of CRP was also found significantly increased (p<0.001) in these patients which shows the presence of inflammation (Table No.13, Graph No.13d).
In our study, we found that some non-diabetic postmenopausal women have developed the knee osteoarthritis which is shown by increased sVCAM-1 level and radiological findings. In these women FBS level is reported non-significant while dyslipidemia is present (Table No.14, Graph No.14a,b) which is same as the study of Hart et al., 1995; Borman et al., 1999; Mishra et al., 2012; Qureshi et al., 2014; Ashraf et al., 2015. The occurrence of dyslipidemia in these non-diabetics may be due to decreased estrogen level (Ashraf et al., 2015) or due to sedentary lifestyle results in the diminution of physical activity (Lipiello et al., 1991; Sturmer et al., 1998). The inflammatory marker CRP was also significantly increased (p<0.01) in these women (Table No.14, Graph No.14d) which is same as quoted by Spector et al., 1997; Tamm et al., 2005; Pearle et al., 2007; in addition to this VCAM-1 was also found highly significantly increased (p<0.001) (Table No.14, Graph No.14e) which is consistent with the study of Schett et al., 2009; Kalichman et al., 2011; Nell-Duxneuner et al., 2013. Osteoarthritis is the most common joint disorder. The pain is the initial and core clinical feature followed by impaired joint mobility, reduction of muscle strength, and loss of joint function (Felson et al., 2006; Lane et al., 2007). The VCAM-1 is a surface sialoglycoprotein that binds α4β1 and α4β7 integrins and mediates the heterotypic cellular aggregation (Cybulsky et al., 1991). This molecule expressed in the endothelium of the knee. The expression of VCAM-1 is controlled by cytokines like IL-1 and TNF-α (Kienzle et al., 1998). The establishment of novel laboratory biomarkers and risk algorithms for the prediction of severe OA is of great interest for several reasons. First, the standard risk factors of age and overweight do not allow accurate risk prediction. Second, OA is a highly prevalent disease necessitating accurate identification of subjects at greater risk of rapid disease progression and development of severe phenotypes. Third, early diagnosis of OA is difficult because
disease onset is gradual and precedes clinical manifestation. These non-diabetic postmenopausal women group increased uric acid suggestive of gouty arthritis which is consistent with the study of Al Arfraj et al., 2003; Ashraf et al., 2015; Ding et al., 2016; Shrivastava et al., 2016 (Table No.14, Graph No.14a). The possible mechanism between high serum uric acid level and knee osteoarthritis may be that higher level of uric acid might lead to the formation of microcrystals in the joint space which is responsible for the commencement of inflammatory chain leading to osteoarthritis (Shrivastava et al., 2016). Martinon et al., 2006 found that monosodium urate crystals can stimulate the release of IL-1β from the NALP3 inflammasome in monocytes which may trigger a powerful inflammatory reaction that contributes to increasing CRP and joint damage and in turn osteoarthritis at last.

Diabetes is known as the low-grade inflammatory disease. Our study is showing that all type 2 diabetic postmenopausal women suffering from knee osteoarthritis having severe hyperglycemia and dyslipidemia (Table No.15, Graph No.15a,b). The same study has been quoted by Nieves-Plaza et al., 2013; Malot et al., 2017; Ahmed et al., 2017. This is because of lack of insulin and the estrogen. The insulin and estrogen both have their potent role in controlling hyperglycemia and dyslipidemia respectively. The level of sVCAM-1 and CRP both found significantly increased (p<0.001) in diabetic postmenopausal women with knee osteoarthritis which is agreement with the study of Schett et al., 2013 (Table No.15, Graph No.15d,e).The increased level of CRP in type 2 diabetic postmenopausal women with osteoarthritis is also in agreement with the findings of Sowers et al., 2002; Engstrom et al., 2009; Oliinyk et al., 2016. These diabetic subjects have severe osteoarthritis. The increased uric acid level is also suggestive of osteoarthritis in these patients (Table No.15, Graph No.15a).
The increased concentration of glucose in diabetic cartilage matrix induce an inflammatory state leads to enhanced cartilage degradation and faster rate of progression of osteoarthritis (Berenbaum et al., 2011). Schett et al., 2013 reported that elevated levels of inflammatory cytokines are responsible for inflammation which is more frequent and severe in the joints of diabetic subjects than non-diabetic subjects. Another concept for hyperglycemia and osteoarthritis suggest that osteoarthritis is degenerative joint disease based on continuous mechanical overload to a metabolic etiopathogenesis. In type 2 diabetes mellitus the alteration in glucose metabolism directly affects the joint integrity independent of body weight. Hyperglycemia has been recognized as a primary factor in the development of diabetic microvascular disease (Ruderman et al., 1992), but the biochemical mechanisms responsible for the toxicity of high glucose concentrations on vascular cell functions and their implication for cell-cell interaction in the microcirculation remain elusive. Data showed that increased intracellular concentration of glucose and glycolytic intermediates trigger acute reversible changes in cellular metabolism leading to increased aldose reductase activity (Ruderman et al., 1992) and to de novo synthesis of diacylglycerol responsible for protein kinase C (PKC) activation (Lee et al., 1989; Porte et al., 1996). The deleterious effects of high glucose on cell function and cell-cell interaction is additionally mediated by advanced glycosylation end products (AGEs) that accumulate in the plasma and vascular tissue of diabetics (Vlassara et al., 1992; Vlassara et al., 1994). AGEs are highly reactive substances resulting from prolonged exposure of proteins to high glucose concentration. They represent a heterologous group of compounds deriving from Amadori product, an early intermediate of the nonenzymatic reaction of glucose with free amino groups of proteins (Ruderman et al., 1992; Bucala et al., 1995). The in vivo significance of
elevated circulating and tissue AGEs as direct contributors to the accelerated vascular disease has been highlighted by in vitro studies showing that AGEs promote monocyte migration (Kirstein et al., 1990; Schmidt et al., 1993) and production of cytokines (Vlassara et al., 1988) and growth factors (Kirstein et al., 1990). These effects are mediated by interaction with a specific receptor, RAGE, a newly identified member of the immunoglobulin superfamily expressed on mononuclear phagocytes as well as vascular smooth muscle cells and endothelial cells (Neeper et al., 1992; Vlassara et al., 1992; Brett et al., 1994). In endothelial cells in culture, AGEs induce cellular oxidant stress thereby increasing vascular permeability (Vlassara et al., 1992) and upon binding to RAGE, activate the transcriptional factor NF-κB (Schmidt et al., 1995). Advanced glycation end products may also participate in diabetes-induced osteoarthritis. They are known to accumulate in cells and tissues under high glucose concentration. In vitro, AGEs induced a proinflamatory and procatabolic phenotype of chondrocytes via RAGE and toll-like receptor. The activation of these receptors in chondrocytes induced a decrease of PPAR-γ and an activation of NF-κB and MAP-kinase pathways (Rasheed et al., 2011; Rasheed et al., 2012; Chen et al., 2013; Wang et al., 2016). The experimental evidence is available that expression of adhesive molecules is critically dependent on intracytoplasmic activation of NF-κB. Thus specific subunits of NF-κB differentially regulate gene expression of E-selectin, ICAM-1 and VCAM-1 in endothelial cells activated by inflammatory cytokines (Shu et al., 1993; Read et al., 1994; Ledebur et al., 1995). Morigi et al., 1998 showed that specific inhibitor of NF-κB significantly reduced high glucose-induced leukocyte adhesion can be taken to suggest that hyperglycemia promoted upregulation of adhesive molecules through NF-κB activation. The shedding of sVCAM-1 is consistent with proteolytic cleavage at a site close to the point of membrane insertion.
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Shedding of adhesion molecules from cell surface may provide a means of regulating cell adhesion (Pigott et al., 1992). Soluble VCAM-1 may be chemotactic for T lymphocytes and monocytes and may promote angiogenesis (Koch et al., 1995; Kitani et al., 1998; Tokhuria et al., 2000).

In this study, the second age group was between 61-75 years in both diabetic and non-diabetic postmenopausal women. Among the older adults, the pain intensity and pain interference are more common in women. The prevalence of chronic pain is higher in older than in younger adults (McCarthy et al., 2009). Pain is commonly measured by separately assessing pain intensity and its functional consequences (Jensen et al., 2001; Johannes et al., 2010). The pain interference as reflected by measures of activity limitations and health-related qualities of life increases with age (Thomas et al., 2004). The risk factors for pain in older adults include the female gender and obesity (Leveille et al., 2005; Shi et al., 2010). The knee pain often the most prominent and disabling symptom of knee osteoarthritis and is common among the older adults. In our study, we found that in group IIA (2) postmenopausal women there was no change in the biochemical parameters though the number of subjects in this category was very less n=13 (Table No.16) hence no statistical analysis could be made but these observations suggest that no change is in this category among biochemical parameter but in diabetic group IIIA (2) of same age the mean level of all the parameters were found increased but the statistical analysis again could not be carried out because of very less number of subjects n=5 (Table No.17).

Further, in this study, we found that among the elderly postmenopausal women the majority of women have radiological findings with knee pain. In group IIB (2), except FBS and HbA1c all parameters showed the significant rise (Table No.18; Graph No.18a-e). The knee osteoarthritis or pain in knee associated with BMI also
The degenerative concept of osteoarthritis view changes in osteoarthritis as process of wear and tear. Long time usage and exaggeration will wear out the joint. Following the recurring process, osteophyte was formed as response of bone reparation. This concept is supported by an imbalance between clinical manifestation (pain) and instability of joint with the broken structure of joint and radiographic changes (Kertia et al., 2005). Soran et al., 2008 reported that levels of serum high-density lipoprotein-cholesterol (HDL-C), total thiol (total free sulfhydryl groups, −SH), paraoxonase and arylesterase activities were significantly lower in the osteoarthritis patient group than in controls, while lipid hydroperoxide (LOOH) and low-density lipoprotein (LDL) levels were significantly higher. Dyslipidemia may result in the ectopic deposition of lipids in particular in chondrocytes, which exacerbate lipid metabolism disorders in degenerative articular cells and progress osteoarthritis. Thus obesity/ BMI related metabolic factors, especially adipokines, can induce the expression and release of inflammatory factors and metabolic enzymes, inhibit the synthesis of articular cartilage and stimulate the remodeling of subchondral bone (Zhuo et al., 2012). Singh et al., 2002 found that patients with OA are more likely to have hypertension (40 % vs. 25 %), high total cholesterol (32 % vs. 24 %), and renal impairment (37 % vs. 27 %) compared with the unaffected population. The mechanism may involve ischemia below the cartilage of knee osteoarthritis patients caused by hypertension. This type of ischemia can inhibit the metabolism of articular cartilage and trigger bone remodeling. This is same as we have observed in our study. The hyperlipidemia is a sign of disorder of lipid metabolism, which could disturb the balance between lipid and bone or cartilage metabolism. Adipocytes share a mesenchymal stem cell precursor with osteoblasts and chondrocytes (i.e. musculoskeletal cells) (Pittenger et al., 1999). Elevated lipids level up-regulate
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proliferation of adipocytes, which could competitively inhibit proliferation and differentiation of musculoskeletal cells. Zhou et al., 2017 reported the hyperlipidemia and its association with clinical knee osteoarthritis. Moreover Fox et al., 2007 reported that elevated serum TG as one index of metabolic disorder following central obesity was strongly associated with knee osteoarthritis. Aspden et al., 2001 reported that elevated lipids level might implicate the formation of musculoskeletal cells from their stromal precursors. The deposition of lipid in the cartilage reduces blood flow and then leads to ischemia and ultimately the bone dystrophy among non-diabetics (Ghosh et al., 2001; Dore et al., 2012). The inflammatory factors might partly explain the association with hyperlipidemia. The increase in the fatty acid and cholesterol crystals, possibly activating the Nalp3-inflammasome and leading to increased release of IL-1β which is involved in the progression of knee osteoarthritis in non-diabetic subjects (Duewell et al., 2010; Joosten et al., 2010; Snodgrass et al., 2013). CRP is one of the useful markers of systemic inflammation. The increased level of CRP in our study among non-diabetic with radiological changes may be indicative of the onset of osteoarthritis. This is same as quoted by Sharif et al., 2000; Takahashi et al., 2004; Tata et al., 2012; Pirdawood et al., 2017. Now, in this group, the significant rise in the CRP constantly leads to stimulate the adhesion molecule i.e. sVCAM-1. In this group IIB (2) the increased level of VCAM-1 with knee osteoarthritis is present which is in line with Hoeven et al., 2015. Schett et al., 2009 suggested that VCAM-1 has an independent role in the prediction of severe knee osteoarthritis although the pathophysiological role is not unclear. A molecular study involving mesenchymal stem cells in human articular cartilage has suggested the role of VCAM-1 in the osteoarthritis process (Grogan et al., 2009). A recent study of the knee osteoarthritis patients showed a non-mechanical link with cartilage volume loss, an early feature of
knee osteoarthritis in obese women (Berry et al., 2011). VCAM-1 has been shown to mediate the interaction of chondrocytes with immune cells and could thus by itself contribute to immune-mediated cartilage damage in osteoarthritis (Kienzle et al., 1998). Further, in the group IIIB (2) diabetic postmenopausal elderly women showed that all biochemical parameters were increased significantly (p<0.001) as compared to control group (Table No.19; Graph No.19a-e). This is same as quoted by Eymard et al., 2015; AL-Rubiae et al., 2011. Diabetes is the major risk factor for knee osteoarthritis among the elderly postmenopausal females. Diabetes develops a more severe clinical symptom of osteoarthritis and structural joint damage in terms of joint effusion and synovitis in ultrasonography (Schett el al., 2013). He further predicted the development of severe osteoarthritis independent of age and BMI. Wen et al., 2013 reported severe articular cartilage damage in type 2 diabetes patients with hypertension as compared to control subjects. Rosa et al., 2009 reported that chondrocytes of osteoarthritis exposed to high glucose concentration exhibit impaired glucose transporter-1 downregulation. So, the impaired glucose transporter-1 downregulation may contribute to an important pathogenic mechanism by which conditions characterized by hyperglycemia may promote degenerative changes in chondrocytes, facilitating osteoarthritis progression. In diabetes due to formation of advanced glycation end products which are supposed to play a role in the development of osteoarthritis of knee (Verzijl et al., 2003; DeGroot et al., 2004; Zhang et al., 2017). AGE formation could contribute to osteoarthritis through effects on the mechanical properties of cartilage as well effects on the cells. Modification of collagen by AGE formation results in increased cross-linking of collagen molecules. The most common AGE-related cross-link is pentosidine which has been found to be present in cartilage in increasing amounts with the increase in age (Bank et al., 1998;
Verzijl et al., 2000; Verzijl et al., 2002). Formation of excessive collagen cross-links affects the biomechanical properties of cartilage resulting in increased stiffness making the cartilage more brittle (Chen et al., 2002) and increasing the susceptibility of the tissue to fatigue failure (Bank et al., 1998). Increased levels of AGEs in cartilage have also been associated with a decline in anabolic activity (DeGroot et al., 1999). In this group III B (2) the patients reported to have hypertension and dyslipidemia also. This may be due to diabetes. The relationship of hypertension and osteoarthritis explained by Wen et al., 2013 that narrow or constricted vessels restrict blood flow to subchondral bone, and impair the circulation and nutrition supply to overlying articular cartilage, and ultimately contribute to the deterioration of cartilage degeneration in osteoarthritis. In elderly postmenopausal women the cause of hyperlipidemia may be the insulin resistance or severe deficiency of estrogen as we have discussed earlier in Table No-15. There were two possible pathways for T2DM involved in the pathogenesis of OA. Firstly, the insulin resistance in T2DM might trigger the cascade inflammatory reactions (Berenbaum et al., 2012), which is known to play an important role in the development of osteoarthritis (Wang et al., 2011). Secondly, the inflammation produces CRP, which, unfortunately, can damage the arteries by helping to form plaque while attempting to tackle a long-term condition like high blood pressure often an accompanying T2DM (Nielen et al., 2012). CRP, in particular, is produced in response to inflammation, infection, and injury, and has been correlated with complications related to conditions such as hypertension, cardiovascular disease, and diabetes (Gabay et al., 1999; Ablij et al., 2002; Dalton et al. 2003; DiNapoli et al., 2003). In relation to osteoarthritis, elevated levels of CRP have been correlated with synovial fluid interleukin (IL)-6 and degree of synovial fluid infiltration (Pearle et al., 2007). The presence of inflammatory synovitis in
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individuals with osteoarthritis, which includes the infiltration of macrophages and lymphocytes, is indicative of more severe pain and joint dysfunction, and may be more predictive of faster rates of cartilage loss in elderly diabetic women (Sellam et al., 2010; Scanzello et al., 2012). The cartilage loss could be detected by estimating sVCAM-1 in diabetic postmenopausal elderly women. We observed the highest level of sVCAM-1 among these elderly women. The mechanism of release of sVCAM-1 as we have already discussed earlier in middle-aged women. The difference between the releases of sVCAM-1 due to cartilage loss is more in elderly women as compared to younger women. Further we compare the mean level of sVCAM-1 according to age wise distribution of subjects in both diabetic and non-diabetic group with radiological findings of knee osteoarthritis. In this the mean levels of sVCAM-1 were found to be highly significant (p<0.001) within and between the age groups (Table No-20). This concludes that sVCAM-1 is associated with cartilage damage and is responsible for osteoarthritis whether the patient is diabetic or non-diabetic.

The correlative studies between sVCAM-1 and other anthropometric and investigated parameters were conducted (Table No. 21-24, Graph No. 21-24) and observed a significant positive correlation of sVCAM-1 with age (p<0.01) in both diabetic and non-diabetic postmenopausal women with osteoarthritis which is same as quoted by Schett et al., 2009. The increase in VCAM-1 with age shows that as the age advances cartilage damage occur at a higher rate. We also found a significant positive correlation of sVCAM-1 with BMI, FBS, HbA1c, TC, LDL-C and significant negative correlation with HDL-C in both diabetic and non-diabetic postmenopausal women showed that obesity, hyperglycemia and dyslipidemia played an important role in the progression of osteoarthritis. CRP also showed a highly significant positive correlation with sVCAM-1(p<0.001) in both diabetic and non-diabetic
postmenopausal women which shows that as the inflammation increases, cartilage damage also increases while a negative correlation of estrogen with sVCAM-1 (p<0.01) shows a protective effect of estrogen on cartilage damage.

The correlative studies and statistical analysis led to conclude that soluble VCAM-1 play role as severity marker for osteoarthritis in middle-aged and elderly women, because as osteoarthritis progress cartilage damage starts, which causes unbearable pain in the knee. In postmenopausal women due to lack of estrogen this severity increases in both diabetic and non-diabetic subjects (women). Therefore, estimation of soluble VCAM-1 is helpful for marking severity of disease in an early phase of osteoarthritis in diabetic/ non-diabetic postmenopausal women. The change in lifestyle, weight reduction may be helpful in reducing the severity of osteoarthritis.