Rheumatoid arthritis (RA) is a chronic, systemic and autoimmune inflammatory joint disease which causes cartilage and bone destruction leading to ultrastructural alterations. Therefore, it is important to study ultrastructural changes for diagnosis of the disease. Osteoarthritis (OA) and RA synovium was obtained from patients undergoing total knee replacement surgery. Electron microscopic blocks were formed for performing transmission electron microscopy (TEM) to study ultrastructural changes in synovium of OA and RA. OA tissues demonstrated mostly the necrosed area, no perivascular infiltration and well stacked collagen fibers. Whereas, RA tissues showed the presence of activated type A synoviocytes, type B synoviocytes, plasma cells and siderosomes. Ultrastructural findings in RA tissue showed severe inflammation than in OA tissues. These observations may play an important role as diagnostic criteria for arthritis.

**Key words:** Osteoarthritis, rheumatoid arthritis, Synovium, Transmission electron microscopy

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disorder which shows the systemic inflammation, persistent synovial inflammation, autoantibodies and multiple proinflammatory cascade causing persistent synovitis, leading to damage in articular cartilage and bone (Scott, et al., 2010), while osteoarthritis (OA) is a degenerative disease and is the most important reason for disability in the elderly population leading to persistent knee pain and functional limitation. Rheumatoid arthritis affects approximately 1% of the world population (Joshi, et al., 2003). Many patients show extra-articular manifestation like ophthalmologic manifestations, rheumatoid nodules, neuropathy, amyloidosis, vasculitis etc. (Turesson, C., et al., 2002) and systemic manifestations such as anaemia, cardiovascular disease (CVD), fatigue, depression, osteoporosis or both (Dayer, et al, 2010, Pollard, et al, 2005). The disease exact etiology is still unclear.

Synovium consist of two layers, synovial lining or intima, which consists of macrophage like synoviocytes (Type A) and fibroblast like synoviocytes (Type B) and other is synovial sublining (Tarner, et al., 2005, Barland, et al 1962, Wilkinson, et al., 1992). In RA, synovial sublining is heavily infiltrated with various cells which includes natural killer cells, immune cells (B cells and T cells), mast cells, neutrophils and dendritic cells resulting in thickening of synovial membrane. Increase in cellularity leads to local hypoxia condition resulting in angiogenesis and release of vascular endothelial growth factor (VEGF) and Fibroblast growth factor (FGF), the pro-angiogenic factors by macrophages (Paleolog, et al., 1998, Fava, et al., 1994, Paleolog, et al., 2002). The articular cartilage is invaded because of the increased cellularity of synovium and leads to formation of pannus, which is rich in osteoclasts and fibroblasts. The present study focused on studying ultrastructural alterations in synovium of rheumatoid arthritis and osteoarthritis. This study also investigated collagen fibers, several cells involved in processes of inflammation and interaction of active inflammatory cells with synovial cells through Transmission electron microscopy. Detailed ultrastructural analysis of the synovium in rheumatoid arthritis and osteoarthritis may help to understand the pathophysiology and clinical manifestation of diseases like rheumatoid arthritis and osteoarthritis.

**MATERIALS AND METHODS**

The present study is a comparative study between rheumatoid arthritis and osteoarthritis at ultrastructural levels. It was done on synovium which is being collected after total knee replacement surgery of the diagnosed cases of OA and RA with their due consent and after getting ethical clearance from the Institute Ethical Committee, JMI, New Delhi. The diagnosis for osteoarthritis was performed as per revised classification criteria's. (Joshi et al 2003) and the diagnosis of rheumatoid
arthritis was done as per revised 2010 ACR/ Eular classification criteria's (Aletaha, et al., 2010).

To visualize the ultrastructural changes in the synovium of 20 cases each of OA and RA in the study was done by Transmission electron microscopy. The synovial tissue (1mm³) were fixed in modified Karnovsky’s fixative (2.5% glutaraldehyde + 2% paraformaldehyde, prepared in 0.1M phosphate buffer, pH 7.4) at 4°C overnight and were washed thoroughly 4-5 times in 0.1M PB on the subsequent day. Further, the tissue blocks were post-fixed in 1% Osmium tetraoxide (OsO₄) for 2 hrs. at 4°C and then washed thoroughly 4-5 times in 0.1M Phosphate buffer. The tissues were further dehydrated through ascending grades of acetone at 4°C After dehydration, the tissues were cleaned by immersing in toluene for 30 minutes (2 times) followed by embedding in BEEM capsules by araldite. Through ultrathin microtome, ultrathin sections of 60-90nm thickness were cut and placed on copper grids. The staining of these sections was done by Uranyl acetate (5-10 min) and then washed with graded ethanol and DDW. Further, counter stained by lead citrate for 2-5 min, followed by washing with 0.02M sodium hydroxide and DDW. The stained tissue were viewed under TEM at 80-120kV.

RESULTS AND DISCUSSION

The ultrastructure electron micrograph of synovium (TEM) of OA showed absence of perivascular infiltration (Fig.-1a) and presence of less number of cells with intact collagen fibers (Fig.-1b). Whereas, in the synovium of RA, collagen fibers were phagocytosed by type A synoviocytes demonstrating the autoimmunity in RA (Fig.-2a), type A synoviocytes (Fig.- 2b) and activated type A synoviocytes (Fig.-2c), type B synoviocytes (Fig.-2d) and presence of dark stained siderosomes (Fig.-2e) were also found. Moreover, plasma cells with well developed endoplasmic reticulum and Russell’s bodies as secretary vesicles (Fig.-2f) were also observed in RA.

In the present study, ultrastructural changes were observed by TEM. The synovium TEM micrograph of OA showed well stacked collagen fibers and no perivascular infiltration, while TEM micrograph of RA demonstrated the presence of phagocytosis of collagen fibers by type A synoviocytes, which usually feed upon RBC, cell debris and fibrin (Ghadially, et al., 1967). The absence of type A synoviocytes or macrophage like synoviocytes in the synovium in macrophage-deficient mice (osteopetrotic op/op mice) shows that type A cells are of macrophagic origin (Naito, M., et al., 1991). We had also observed the presence of type A synoviocytes, type B synoviocytes, B cells and plasma cell with well developed rough ER and juxta-nuclear golgi complex along with presence of Russell bodies, which is a RA associated feature (Ghadially, et al., 1997). The presence of little RER and prominent golgi Complex in type A synoviocytes was also shown by (Wyllie, et al., 1964, Roy, et al., 1967, Shannon, et al., 1971) and well

![Fig.1: Electronmicrograph of OA synovium showing (a) No infiltration in blood vessel, scale bar=1µm and (b) Intact collagen fibers in OA, scale bar=1µm.](image-url)
Fig. 2: Electronmicrograph of RA synovium showing (a) Phagocytosis of collagen fibers by type A synoviocytes, scale bar = 500 nm (b) type A synoviocytes, scale bar = 1 µm (c) activated type A synoviocytes, scale bar = 1 µm (d) type B synoviocytes, scale bar = 2 µm (e) presence of dark stained siderosomes, scale bar = 0.5 µm and (f) plasma cells with well developed endoplasmic reticulum and Russel’s bodies as secretary vesicles, scale bar = 1 µm.
developed golgi complex was demonstrated in type B cell than in type A cells (Okada, et al., 1981, Graabk, et al., 1982) in rat and mouse. Morris suggested the presence of siderosomes in RA (Morris, et al., 1986), which were also observed in our present study. In our ultrastructural analysis, we observed various cells i.e. plasma cells, type A & type B synoviocytes which plays a vital role in pathophysiology of rheumatoid arthritis.

CONCLUSION

The underlying ultrastructural changes in OA and RA were distinct. The present study has validated ultrastructural changes in synovium of arthritis patients by Transmission electron microscopy. The findings of the present study show that the RA tissues show severe and widespread inflammation whereas OA exhibits less signs of inflammation. These comparative ultrastructural changes may be implicated for diagnostic criteria for osteoarthritis and rheumatoid arthritis.

Acknowledgements: DB would like to acknowledge Department of Biotechnology, JMI, Delhi for providing infrastructure facilities for research work and authors would also acknowledge Centre for Nanoscience and Nanotechnology, JMI, Delhi for providing TEM facilities. First author acknowledge UGC and JMI for providing UGC Non-Net Fellowship.

Conflict of interest statement: No conflict of interest.

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