Research Article

Comparative Histological Study of Synovitis in Rheumatoid Arthritis and Osteoarthritis

Alzahraa A. Abdelhakeem*, Entesar A. Saber*, Soha A. Abdelwahab*, Nashwa F. Gamal*, Rehab A. Refai* and Petri Lehenkari**

* Department of histology, Minia faculty of medicine, Minia University.

**Medical Research Center Oulu, Department of Surgery, Cancer and Translational Medicine, Oulu University Hospital and University of Oulu, Finland

Abstract

This study is concerned with the detailed description of histological features of the synovial membrane in rheumatoid arthritis (RA) compared to osteoarthritis (OA). We used synovial tissue samples of twenty patients with OA and RA then stained them with common stains to be visualized under light microscopy. Intimal hyperplasia, sub-intimal cellularity, inflammatory infiltration and neo-angiogenesis are evident in RA but mild in OA. Macrophages, B, T lymphocytes and dendritic cells were obviously increased all over the synovium, especially at RA samples. However the presence of pannus and fibrinoid necrosis were characteristic to RA synovitis. We concluded that histology is the gold standard for the diagnosis of synovial lesions and proved to be useful in disease severity diagnosis.

Key Words: rheumatoid arthritis, osteoarthritis**,** synovial tissue

---

Introduction

Joint diseases cause serious medical problems and affecting the life style of several million people world-wide, therefore the world health organization has designated the last decade as the decade of bone and joint according to (Popko et al., ٢٠١١). Rheumatoid arthritis (RA) is distributed universally and definedby Kourilovitch et al., (٢٠١٤) as a systemic chronic inflamma-tory disease of unclear aetiology that is manifested in by a progressive and destructive poly-arthritis in association with serological evidence of auto-reactivity. According to recent review, the annual incidence of RA has been reported to be around ٤٠/١٠٠,٠٠٠ worldwide, being women ٢:١ to ٣:١ more likely to be affected than men.Prevalence also rises with age (Viatte et al., ٢٠١٣). RA is a combi-nation of genetic and environmental factors that when present increase the susceptibility to develop clinical manifestations; ٥٠٪ of the risk for development of RA is attributable to the genetic factors. The environmental risk factors associated with RA are mainly smoking and alcohol intake, increasing the risk up to ٤٠ times compared with unexposed (Liao et al., ٢٠٠٩).

Dieppe, (٢٠٠٠) stated that osteoarthritis (OA) is the most common, and increasingly prevalent, human joint disorder. Clinical and epidemiological studies on OA have recognized a series of etiologic factors including local factors (such as malformations or joint injuries) and systemic factors (such as overweight, race, gender, or metabolic diseases). OA is associated with a loss of proper balance between synthesis and degradation of the macromolecules that gives articular cartilage its biome-chanical and functional properties. Concomitantly in OA, changes occur in the structure and metabolism of the synovium and subchondral bone of the joint as described by Popko and companions, (٢٠١٢).

The synovium is consisting of lining intima and sub-intimal layer. The intima is formed mainly by fibroblast-like cells and macrophage-like cells. The sub-intimae showed different types of connective tissue: areolar, adipose and fibrous. Synovitis is a major characteristic of chronic inflammatory joint diseases of autoimmune origin. Studies in RA indicate that the synovial membrane has a dominant role in the joint inflammation and destruction (Baeten et al., ٢٠٠٠); hence the synovium was the scene of our study where the joint damage begins. Increasing knowledge about histo-pathological processes in inflammatory joint diseases is needed to initiate personalized medicine based on targeted treatments in the future. Although OA is considered a non-inflammatory condition, it is widely accepted that synovial inflammation is a feature of it. However, there is no role of immune cells in OA (De Lange-Brokaar et al., ٢٠١٢).

Patients and methods

Patients and samples

The study used synovial tissue samples from twenty patients, with RA or OA. Biopsies were obtained by knee replacement surgery at Oulu university hospital. All RA patients fulfilled the diagnostic criteria of the American College of Rheumatology for RA (١٩٨٧ or ٢٠١٠) (Aletaha et al., ٢٠١٠). Osteoarthritis of the knee was diagnosed on the basis of X-ray and clinical examination. They were divided as follow:

- Group ١ (OA): consisted of six samples.
- Group ٢ (RA): consisted of fourteen samples

Methods and chemicals:

Histological staining with Hematoxylin and Eosin (H & E) was performed using paraffin sections, according to Bancroft et al., (٢٠١٣). After collection, the synovial tissue samples were cut into small pieces, fixed in ١٠٪ formalin in phosphate-buffered saline (PBS) at room temperature for ٢٤-٣٦ hours. After proper fixation the samples were automatically processed, embedded in paraffin and cut by automated microtome. ٥µm sections were mounted on glass slides and deparaffinised to be stained. The dewaxed sections were put in Hx stain for ٦ minutes, washed well in running tap water for ١٥ minutes, then put in eosin for ٧ minutes and the surplus stain was washed off in water. The section were dehydrated in alcohol, cleared in xylene and then mounted on glass slides. The slides was examined via the light microscopy.

Results

The study was conducted to detect the detailed histological changes in the synovium of (RA) patients in comparison to (OA) patients as following:

- **Osteoarthritis group:**

The synovium exhibited no or slight intimal enlargement, where the intimal cell layers were ranging from two to three layers in thickness. The sub-intima showed no or minimal cellular proliferation, with few scattered small blood vessels and inflammatory cells (Fig. ١A & B).

- **Rheumatoid arthritis group:**

In most of the rheumatoid arthritis patients, the synovium consisted of hyperplasic intima reaching five or six layers thick. Lymphocytes sometimes infiltrate the intima (Fig. ٢).The sub-intima showed infiltration with different cell types. Chronic inflammatory cells such as lymphocytes and plasma cells predominated (Fig. ٣A). Macrophages and dendritic cells were also frequently noticed (Fig. ٣B & ٣C). Some large cells appeared with eosinophilic cytoplasm and multiple nuclei (Fig. ٣D). These inflammatory cells were either scattered individually in the sub-intima or in dense confluent inflammatory infiltration that nearly filled up the synovial tissue in some cases and migh form finger-like protrusions of inflamed fibro vascular stromath at was covered by hyperplasic intimal cells (Pannus) (Fig. ٤A). It may encroach on contiguous cartilage and

subchondralbone. The inflammatory cells were also found in small or large lymphatic aggregations especially in the perivascular regions (Fig. ٤B). The synovium of many cases was thrown into papillae (Fig. ٥A). the sub-intima displayed increased cellularity and neoangiogenesis (Fig. ٥B). Some areas of the synovium showed fibrinoid necrosis, characterized by the presence of an amorphous eosinophilic material similar, in morphology, to fibrin within the area of cell death. These areas were surrounded by cellular palisade which is a densely packed layer of cells which tend to be arranged radially (Fig.٦A). Other areas also exhibited vacuolation and degeneration of some sub-intimal cells (Fig. ٦B).

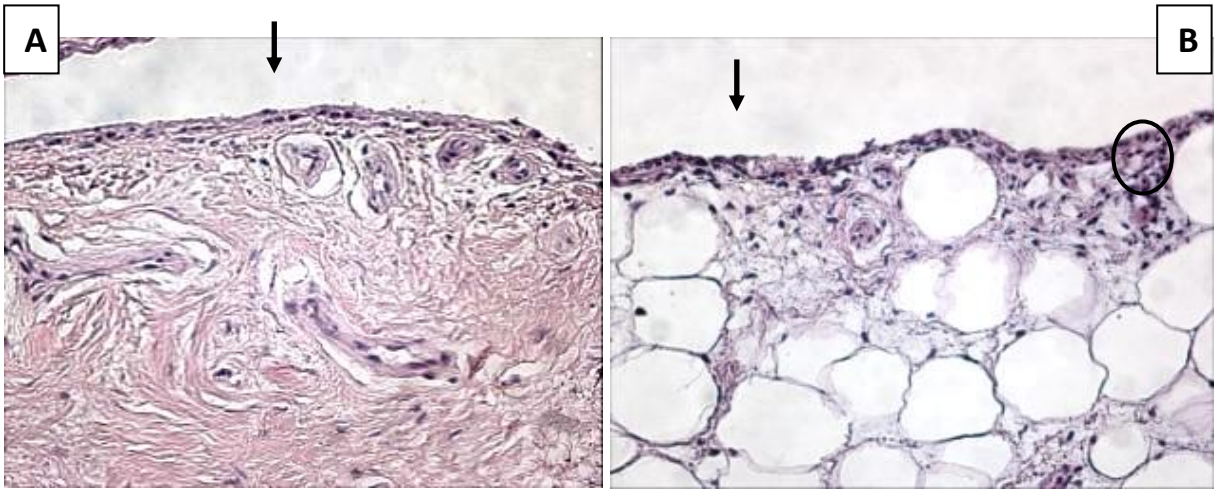

Fig. ١: Photomicrographs showing histological features of OA synovitis, few intimal layers (arrows) with slight stromal cellularity and rare **(A)** or few **(B)** inflammatory cells scattered in the sub-intima (circle).H&E x ٢٠٠

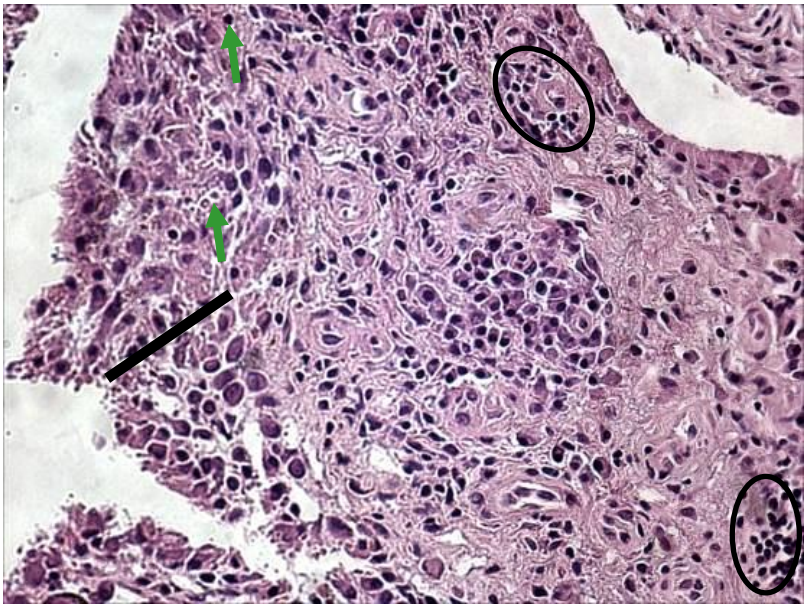

Fig. ٢: Photomicrograph of RA synovial fold showing histological characteristics of RA synovitis, hyperplasia of intimal synoviocytes (spanned by line), with some infilterating lymphocytes at the intima (green arrows). Chronic inflammatory cells are heavily infiltrating the sub-intima (circles).H&E x ٢٠٠

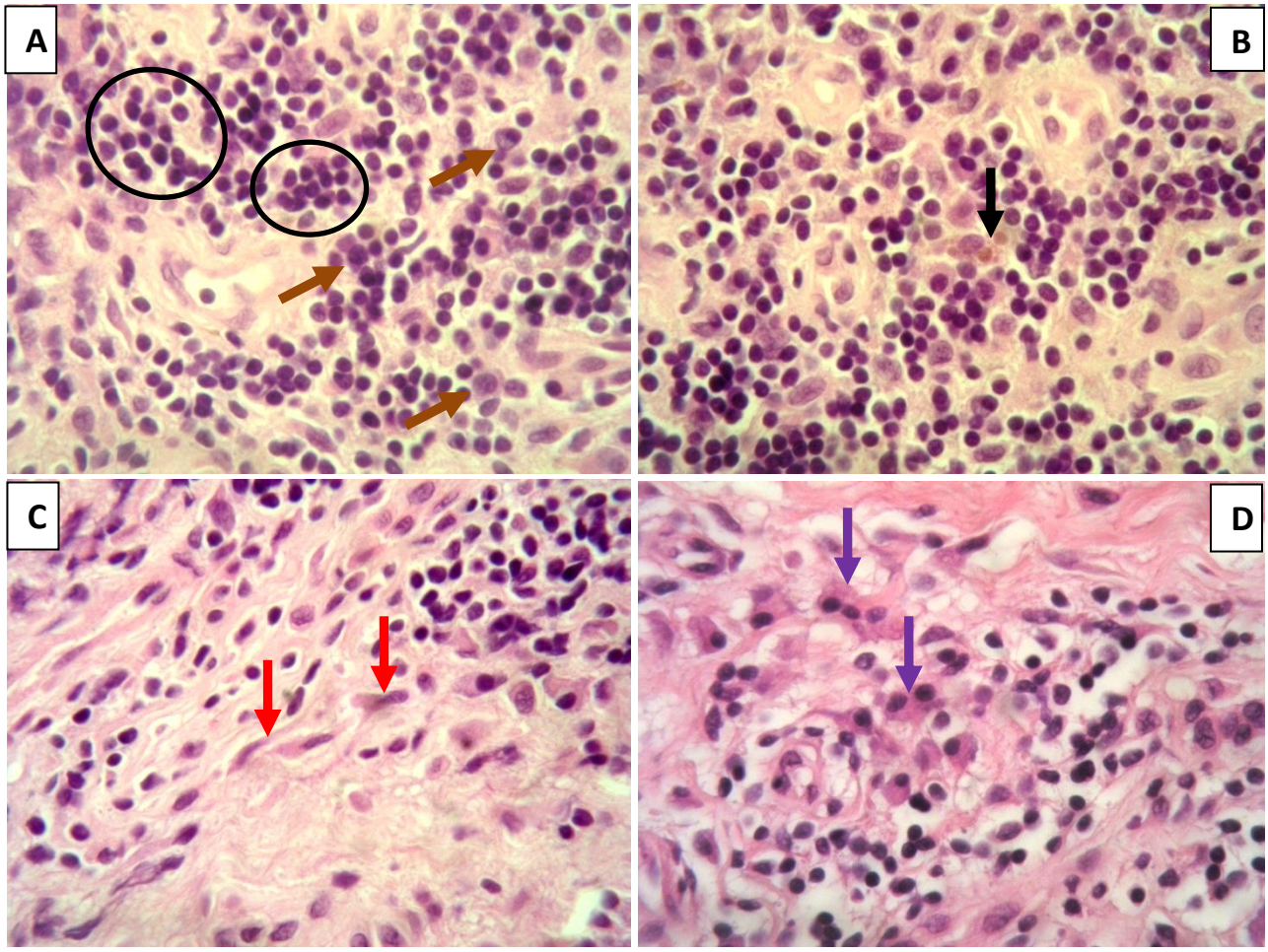

Fig. ٣: Photomicrographs of RA synovia showing many cell types at the sub-intima, **A)** Showing chronic inflammatory cells infilteration (brown arrows indicate plasma cells and circles surrounds infilterating lymphocytes). **B)** Macrophages (black arrow) were seen in between the heavy infiltration of chronic inflammatory cells. **C)** Dendritic cells were noticed (red arrows). **D)** Giant cells were also detected (violet arrows). H&E x١٠٠٠

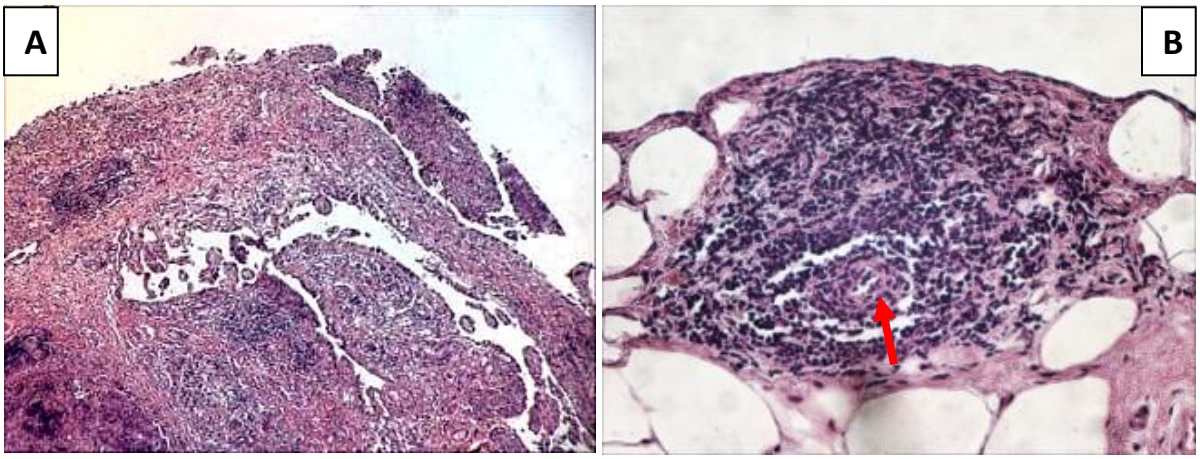

Fig. ٤: Photomicrographs of RA synovitis showing **A)** finger-like protrusions of highly inflamed fibro vascular stroma covered by intimal cells (pannus formation), **B)** showing large inflammatory aggregation around blood vessels (arrow). H&E, A x ٤٠ and B x ٢٠٠

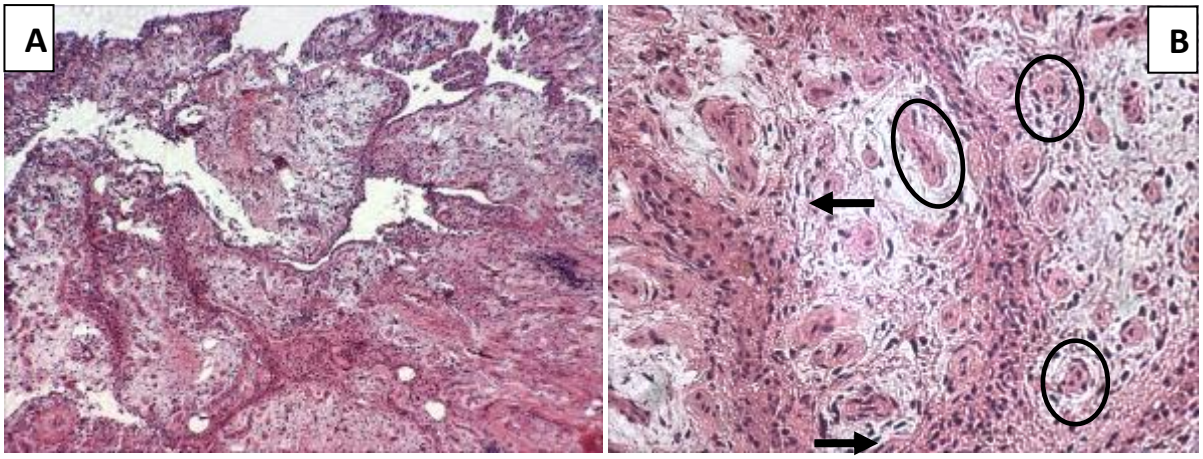

Fig. ٥: **(A)** Photomicrograph of RA synovitis showing the synovium thrown into many papillary like projections. **(B)** Higher magnification of the sub-intima showing fibroblastic proliferation (arrows) and large numbers of small blood vesseles; neoangiogenesis (circles). H&E, A x ٤٠ and B x ٢٠٠

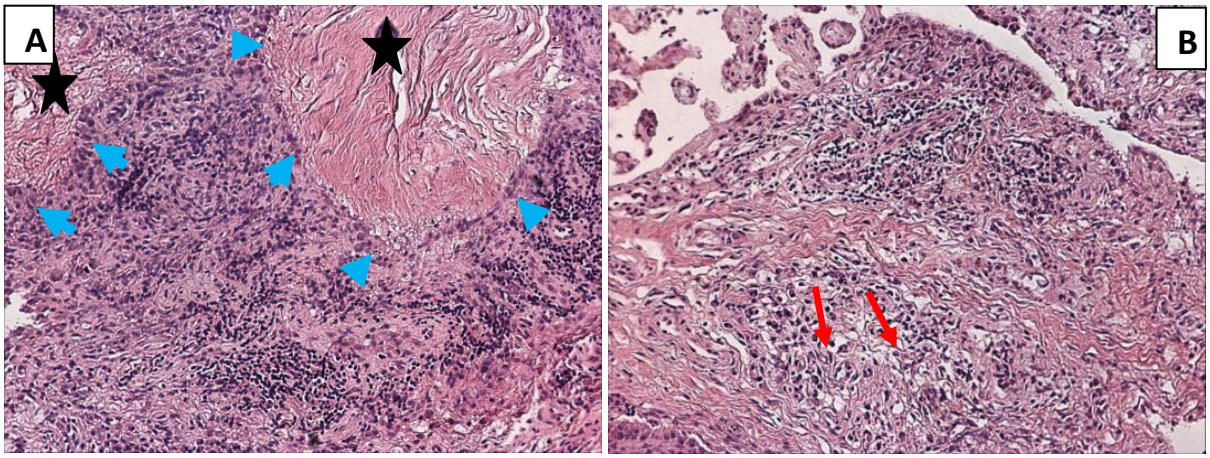

Fig. ٦: Photomicrograph of RA synovitis showing **(A)** Areas of fibrinoid necrosis (stars) surrounded by palisading cells (arrow heads). **(B)** Degenerated or vacuolated cells scattered in the sub-intima (red arrows). H&E x ١٠٠

Discussion

In accordance to Van-de Sande & Baeten (٢٠١٥), the current results exhibited hyperplasia of the synovial intima of RA patients that was frequently thrown into papillae. The intima also displayed infiltration with some inflammatory cells. Their study attributed this hyperplasia to accumulation of macrophages and proliferation of fibroblast-like synoviocytes. In addition, Bartok & Firestein (٢٠١٠) confirmed these intimal alterations and reported reduction of apoptosis in fibroblast-like synoviocytes as another cause of intimal hyperplasia.The sub-intima displayed increased cellularity and neo-angiogenesis, in line with Elshabrawy et al., (٢٠١٥) who reported that angiogenesis, a feature from the earliest stages of RA, plays a critical role in the pathogenesis of several inflammatory autoimmune diseases. Their study also founda vast influx of inflammatory cells such as B, T lymphocytes, plasma cells, macrophages and dendritic cells in the synovial sub-intima of RA samples. These infiltrating leucocytes produce a vast amount of pro-inflammatory and destructive mediators that contribute to synovitis as well as to cartilage and bone destruction. Few large multinucleated cells, noticed at sub-intima of

our RA samples, may be a result of fusion of macrophages forming giant cells or osteoclast like cells. This was agreed by Nevius and coworkers, (٢٠١٥). Our results described distribution of these inflammatory cells in RA synovial tissues sub-intima as individually scattered, peri-vascularly aggregated or densely distributed all over the sub-intima. This was in agreement with Van-de Sande & Baeten (٢٠١٥) who proposed a pathophysiological relevance of these different forms. One of the hallmarks observed in RA samples of this study, is the finger-like protrusion of inflamed fibro vascular stroma covered by hyperplasic intima, or as named by authors Pannus. This finding was describedbyRobbins et al., (٢٠١٠) as osteoclast-rich portion of the thickened hyperplastic synovial membrane.

Pannus may encroach on contiguous cartilage and sub-chondral bone causing bone resorption and destruction. Also enzymes secreted by its synoviocytes, erode and degrade articular cartilage. Other characteristic finding, in RA samples, was the appearance of areas of fibrinoid necrosis characterized by the presence of an amorphous eosinophilic material, within the area of cell death, surrounded by a densely packed palisading layer of cells, mainly histocytes and fibroblasts (Cojocaruet al, ٢٠١٠). Although neglected in the recent literature, the results of this work have noticed the emergence of vacuolation and degeneration at sub-intimal cells in some RA samples. Since long, a study of Ishikawa & Ziffin (١٩٧٦) had reported marked degeneration of fibro-blasts in the vicinity of lymphocytes at the peri-vascular infiltrates of the rheumatoid synovium.

The current study used OA group of patients as a disease control. In contrast to RA, the present study showed minimal histological changes at the synovium of OA patients what was also confirmed by Wenham & Conaghan, (٢٠١٠). Taken together, all these observations suggest that the synovial membrane in RA is both the primary site of inflammation, triggered by inflammatory cells, and the main effector organ as the hyperplastic pannus leads to cartilage and bone erosion.

References

١. Aletaha, D., Neogi, T., Silman, A. J., Funovits, J., Felson, D. T., Bingham, C. O., ٣rd, et al. (٢٠١٠). ٢٠١٠ rheumatoid arthritis classification criteria: an American College of Rheumatology/European League against Rheumatism collaborative initiative. Ann Rheum Dis, ٦٩(٩), ١٥٨٠-١٥٨٨.

٢. Baeten, D., Demetter, P., Cuvelier, C., Van Den Bosch, F., Kruithof, E., Van Damme, N., et al. (٢٠٠٠). Comparative study of the synovial histology in rheumatoid arthritis, spondyloarthropathy, and osteoarthritis: influence of disease duration and activity. Ann Rheum Dis, ٥٩(١٢), ٩٤٥-٩٥٣.

٣. Bartok, B., & Firestein, G. S. (٢٠١٠). Fibroblast-like synoviocytes: key effector cells in rheumatoid arthritis. Immunol Rev, ٢٣٣(١), ٢٣٣-٢٥٥.

٤. Cojocaru, M., Cojocaru, I. M., Silosi, I., Vrabie, C. D., & Tanasescu, R. (٢٠١٠).Extra-articular Manifestations in Rheumatoid Arthritis.Maedica (Buchar), ٥(٤), ٢٨٦-٢٩١.

٥. De Lange-Brokaar, B. J. E., Ioan-Facsinay, A., van Osch, G. J. V. M., Zuurmond, A. M., Schoones, J., Toes, R. E. M., et al. (٢٠١٢). Synovial inflammation, immune cells and their cytokines in osteoarthritis: a review. Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society, ٢٠(١٢), ١٤٨٤-١٤٩٩.

٦. Dieppe, P. (٢٠٠٠). The management of osteoarthritis in the third millennium. Scand J Rheumatol, ٢٩(٥), ٢٧٩-٢٨١.

٧. Elshabrawy, H. A., Chen, Z., Volin, M. V., Ravella, S., Virupannavar, S., &Shahrara, S. (٢٠١٥). The pathogenic role of angiogenesis in rheumatoid arthritis.Angiogenesis, ١٨(٤), ٤٣٣-٤٤٨.

٨. Ishikawa, H., & Ziff, M. (١٩٧٦).

Electron microscopic observations of immunoreactive cells in the rheumatoid synovial membrane. Arthritis and rheumatism, ١٩(١), ١-١٤.

٩. Kourilovitch, M., Galarza-Maldonado, C., & Ortiz-Prado, E. (٢٠١٤).Diagnosis and classification of rheumatoid arthritis. JAutoimmun, ٤٨-٤٩, ٢٦-٣٠.

١٠. Liao, K. P., Alfredsson, L., &Karlson, E. W. (٢٠٠٩). Environmental influences on risk for rheumatoid arthritis. Curr Opin Rheumatol, ٢١(٣), ٢٧٩-٢٨٣.

١١. Nevius, E., Gomes, A. C., & Pereira, J. P. (٢٠١٥). Inflammatory Cell Migration in Rheumatoid Arthritis: A Comprehensive Review. Clin Rev Allergy Immunol.

١٢. Popko, J., Guszczyn,T., Olszewski, S., Zwierz, K. (٢٠١٢). Lysosomal Glycosidases in Degradation of Human Articular Cartilage. In D. A. Lemmey (Ed.), Rheumatoid Arthritis - Etiology, Consequences and Co-Morbidities (pp. ٩٧-١١٠): intech.

١٣. Popko, J., Olszewski, S., Guszczyn, T., Zwierz, K., & Pancewicz, S. (٢٠١١). Glycoconjugate markers of joint diseases. BiochemSoc Trans, ٣٩(١), ٣٣١-٣٣٥.

١٤. Robbins, S. L., Kumar, V., &Abbas, A. K (٢٠١٠). Robbins and Cotran PATHOLOGIC BASIS OF DISEASE (٨th ed.): Elsevier Saunders.

١٥. Bancroft, J. D., Suvarna, S. K., & Layton, C. (٢٠١٣). Bancroft's Theory and Practice of Histological Techniques (٧th ed.). China: Churchill Livingstone, Elsevier.

١٦. Van de Sande, M. G., & Baeten, D. L. (٢٠١٥). Immunopathology of synovitis: from histology to molecular pathways. Rheumatology (Oxford).

١٧. Viatte, S., Plant, D., & Raychaudhuri, S. (٢٠١٣). Genetics and epigenetics of rheumatoid arthritis.Nat Rev Rheumatol, ٩(٣), ١٤١-١٥٣.

١٨. Wenham, C. Y. J., & Conaghan, P. G. (٢٠١٠).The Role of Synovitis in Osteoarthritis.Therapeutic Advances in Musculoskeletal Disease,٢(٦),٣٤٩-٣٥٩.