INTRODUCTION

Osteoarthritis (OA) is the most common form of chronic arthritis. OA can be clinically described as joint pain and reduced mobility, associated radiographically with joint space narrowing (JSN), subchondral bone sclerosis and osteophyte formation ***(Luyten et al., 2009)***.

About half of the world's population, who are 65 years or older, suffers from OA ***(Bijlsma et al., 2011; Li et al., 2013b)***.

In 2008, it was estimated that nearly 27 million people in the USA had some form of OA ***(Lawrence et al., 2008)***. However the prevalence of OA varies greatly according to age, sex and geographical area studied ***(Litwic et al., 2013)***.

Many different factors contribute to the onset and progression of OA, including genetics, lifestyle and joint instability ***(Luyten et al., 2009)***.

OA can arise in any synovial joint in the body, but is most common in the hands, knees, hips, and spine ***(Dieppe and Lohmander, 2005)***.

The knee joint is the most clinically significant site of involvement in primary OA (***Honsawek et al., 2010)***. Knee pain due to OA is the most common cause that brings elderly patients to the physician ***(Arya and Jain, 2013)***

Synovial joint is a complex organ consisting of different tissues including the articular cartilage, the subchondral bone, the joint capsule, synovium and synovial fluid that allow for joint movement with minimal friction and wear. Other soft tissue structures include ligaments, tendons and menisci ***(Neu et al., 2008).***

Healthy joint requires a proper balance between molecular signals that regulate homeostasis, damage, restoration, and remodeling which is determined at the level of individual cells and of the interactions between different tissues in the joint organ e.g. articular cartilage, synovium, and bone ***(Lories, 2008).***

The factors that initiate OA are not well understood, and the course of joint degeneration in OA is variable ***(Felson et al., 2000).*** There is a disagreement on defining the primary trigger of OA with some animal models pointing towards changes in articular cartilage occurring first, while others suggesting underlying bone changes first ***(Luyten et al., 2009)***.

Therefore, there has been increasing attention in order to understand the mechanisms that are driving the disease process. Among these mechanisms, the biology of the cartilage-subchondral bone unit has been highlighted as key in OA, and pathways that involve both cartilage and bone formation and turnover have become prime targets for modulation, and therapeutic intervention ***(Luyten et al., 2009).*** **Wnt/β-catenin** signaling pathway, which is involved in skeletal and joint development, has received attention in OA ***(Guo et al., 2004).***

The role for the **Wnt/β-catenin** pathway has been highlighted in several critical aspects of bone biology, including bone mass regulation as well as in some rheumatic diseases such as rheumatoid arthritis (RA) as well as in OA ***(Daoussis et al., 2010).***

This pathway is regulated by several soluble inhibitors such As Dickkopf-1 (Dkk-1), sclerostin and secreted frizzled related proteins ***(Daoussis and Andonopoulos, 2011)***.

Dkk-1 appears to be the most important biologically, its role as a regulator of joint remodeling in animal models of arthritis was explored by ***Diarra and Coworkers, (2007)*** they documented that bone destructive pattern in a mouse model with RA was reversed by the blockade of Dkk-1 to bone forming pattern of OA, indicating that Dkk-1 is a central regulator of joint remodeling, and may be critical in determining the direction of the diseased joint with increased Dkk-1 levels linked to bone resorption and joint destruction and decreased levels to new bone formation (osteophytes).

It appears that the role of Dkk-1 in OA needs further exploration, especially in humans as data are scarce, and its study enhances our understanding of bone physiology and its disturbance in many pathological conditions such as OA, moreover inhibiting or enhancing the function of Dkk-1 may be a way of altering the process of joint remodeling if needed and can open new chances toward more effective therapeutic approach ***(Daoussis and Andonopoulos, 2011)***.

Radiological assessment is regarded as one of the current methods to evaluate the affected joint; it reflects disease severity by grading the joint degeneration ***(Honsawek et al., 2010).*** The Kellgren-Lawrence grading scale representing disease severity has been the most widely used system ***(Kellgren and Lawrence, 1957).***

However, plain radiography has clear limitations in imaging and directly visualizing articular cartilage and other soft tissues, which are frequently involved with disease progression over the years ***(Meenagh et al., 2007).***

Ultrasound (US) has become one of the most important tools of investigations for rheumatologists, it is considered a safe, accurate, non invasive tool that is readily accepted by patient without any radiation hazards ***(Grassi and Cervini, 1998).***

US has many indications in the assessment of patients with OA. US has high value in demonstrating both structural damage and joint inflammation even in early disease. It has been shown to be a valuable tool in the detection of cartilage lesions, demonstrating not only early cartilaginous irregularities but also progressive thinning and deteriorations in late disease in case of structural damage ***(Iagnocco, 2010).***

Recently, interest has been increasing to use non invasive US for diagnosis of knee OA related changes in articular cartilage ***(Moller et al., 2008; Saarakkala et al., 2012).***

AIM OF THE WORK

- To find out the relation between serum levels of **Dkk-1** and the occurrence of primary OA in the knee and if present.
- To find out its relation to disease severity assessed clinically, radiologicaly and sonographicaly.

CARTILAGE HISTOLOGY AND BIOCHEMISTRY

Normal articular Cartilage

Different types of cartilage tissue are present in the body. Histologically they are classified into hyaline, elastic and fibrocartilaginous cartilage. Articular cartilage is a hyaline cartilage ***(Umlauf et al., 2010).***

Articular cartilage is a specialized avascular and aneural connective tissue that provides covering for diarthrodial joints. It is a load-bearing material that absorbs shock and is capable of sustaining shearing forces ***(Martel-Pelletier and Pelletier, 2010; Ouzzine et al., 2012).***

Development of synovial joints, cartilage and endochondral ossification

The long bones of the vertebrate appendicular skeleton arise from initially continuous condensations of mesenchymal cells that subsequently differentiate, segment and cavitate to form discrete elements separated by synovial joint ***(Moskalewski et al., 2013)***; Figure (1).

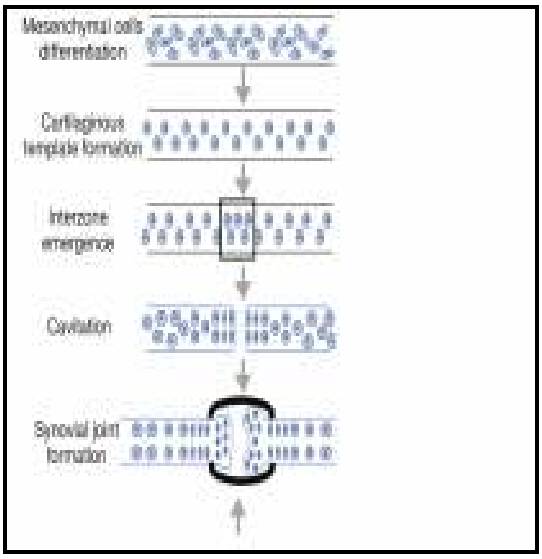

Figure (1): The main steps in synovial joint formation ***(Moskalewski et al., 2013)***.

The mesenchymal cells have the ability to differentiate into cartilage, fat, muscle or bone cells ***(Staines et al., 2012).***

Cartilage development is initiated by chondrogenesis, which is the differentiation of mesenchymal precursor cells into chondrocytes, and is critical for cartilage development ***(Staines et al., 2012).*** Chondrogensis requires mesenchymal condensation and cartilage nodule formation ***(Chun et al., 2008).***

For chondrocyte differentiation, there are four recognized steps involved ***(Staines et al., 2012).***

The initial step of chondrogenesis is the recruitment of mesenchymal chondroprogenitor cells to future sites of skeletal development, followed by cellular aggregation and then

condensation. Finally, differentiation of mesenchymal cells to the chondrogenic lineage with expression of specific extracellular matrix (ECM) molecules such as type IIB collagen and aggrecan to form cartilage tissue ***(Chun et al., 2008).***

Differentiated chondrocytes have two distinct fates, one is to remain as chondrocytes to form articular cartilage and function in joint development. The other fate is maturation into hypertrophic chondrocytes to function as a template for long bone during endochondral ossification ***(Shum and Nuckolls, 2002; Provot and Schipani, 2005)***; Figure (2).

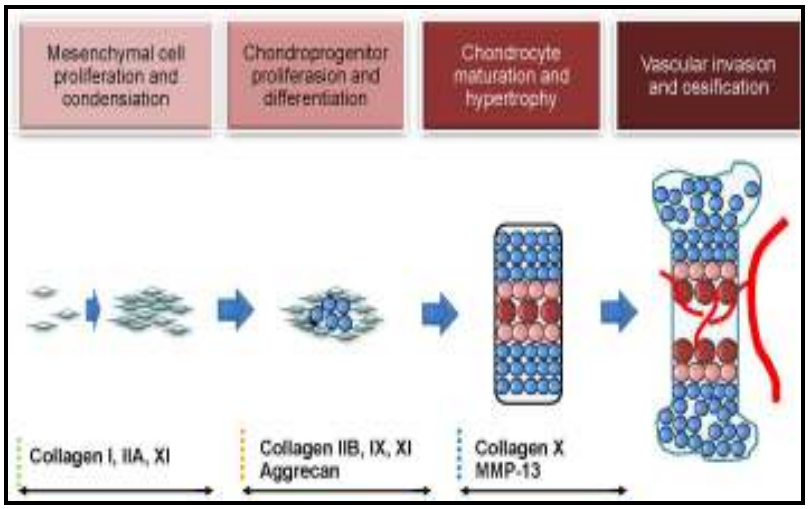

Figure (2): *Cartilage* development and endochondral ossification ***(Chun et al., 2008).***

During endochondral ossification, the size of chondrocyte cells increases after stopping proliferation and the hypertrophic chondrocytes initiate expression of type X collagen and matrix metalloproteinase 13 (MMP-13). Hypertrophic chondrocytes also express vascular endothelial

growth factor (VEGF) to induce attraction for blood vessels in association with other factors, for replacement of cartilage by bone. Hypertrophic chondrocytes are subjected to apoptosis, and the remaining cartilage ECM molecules are replaced by bone matrix produced from osteoblasts ***(Chun et al., 2008).***

According to the established view articular cartilage represents part of cartilage template (Anlage or growth plate) that is not replaced by bone through endochondral ossification. The chondrocytes that form the bulk of the anlage have a transient character and display dynamic phenotype, undergo proliferation, maturation, hypertrophy, and apoptosis in the growth plates, and are finally replaced by bone cells during endochondral ossification which makes it different from the articular chondrocytes which maintain joint function throughout life and do so by retaining a stable and permanent phenotype, producing all the macro- molecular components of articular cartilage ***(Moskalewski et al., 2013).***

However articular chondrocytes can potentially undergo maturation and hypertrophy and display traits of transient growth plate chondrocytes. Such a shift from a permanent to a transient chondrocyte phenotype is often seen in the joints of OA patients ***(Pacifici et al., 2006).***

A number of factors regulating hypertrophic maturation of chondrocytes has been identified, including Wnt signaling pathway. Thus, hypertrophic chondrocytes play an essential role in coordinating chondrogenesis and osteogenesis, as

hypertrophic chondrocytes provide a framework for subsequent bone formation ***(Chun et al., 2008).***

Understanding the mechanisms of chondrocyte differentiation is important for studying cartilage degeneration because similar mechanisms are involved in initiation and progression of the disease ***(Umlauf et al., 2010).***

The structural composition of Articular Cartilage:

Macroscopically, normal hyaline articular cartilage is an unruffled white to yellowish coating the articulating joint surface. It is shiny, elastic and firm. The synovial fluid makes it to appear slippery and provides its gliding properties ***(Aigner and Schmitz, 2011).***

Microscopically, normal cartilage consists of Chondrocytes and ECM which consists of fibrous components mainly collagen type II embedded within amorphous mixture of nonfibrous components mainly proteoglycan (PG) (aggrecan) and water ***(Tanzer, 2006 and Berenbaum, 2008).***

Other non-collagenous and non PG matrix proteins are found in lesser amounts in ECM, they may play a role in integrity of the cartilage e.g Cartilage oligomeric protein (COMP) ***(Goldring, 2013).***

Articular cartilage consists of four zones; the superficial, middle, deep and calcified zone, Figure (3). Within each zone, matrix is divided into 3 regions, the pericellular region, the

territorial region, and the interterritorial region ***(Sophia Fox et al., 2009).***

Structure of articular cartilage zones

Superficial articular cartilage is composed of flattened chondrocytes, and the ECM comprises thin collagen fibrils in tangential array, associated with a high concentration of the small PG "decorin" and a low concentration of " aggrecan " with high concentration of water 75% to 85% and other molecules as Superficial cell protein for joint lubrication also known as lubricin ***(Goldring, 2013).***

Middle layer forms 40% to 60% of cartilage weight, consists of rounded chondrocytes surrounded by thick collagen fibrils in form of radial bundles ***(Goldring, 2013).***

In the deep zone, chondrocytes are grouped into columns or clusters. Collagen bundles are the thickest and are arranged in a radial mode ***(Goldring, 2013).*** Cell density progressively decreases from the surface to the deep zone ***(Stockwell, 1972).***

The last layer of articular cartilage is composed of calcified cartilage with partial mineralization and hypertrophic chondrocytes. The calcified zone is separated from the deep zone by a thick bundle of collagen fibrils termed the tidemark ***(Sharma et al., 2013).***

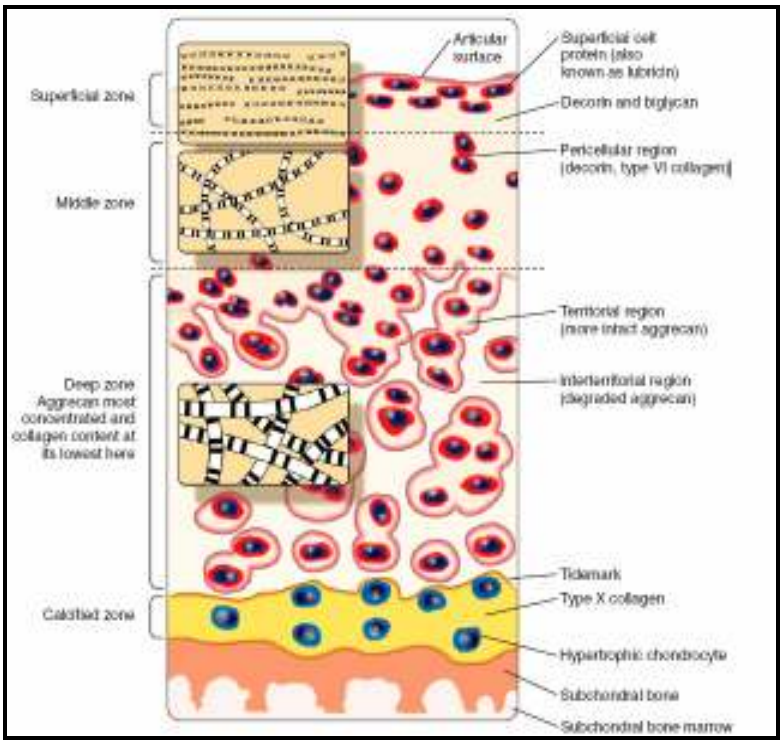

Figure (3): Structure of human adult articular cartilage ***(Goldring, 2013).***

Structure of different Matrix regions:

I) **Pericellular Region:** A thin matrix rim covering chondrocyte's surface, rich in PG and contains type VI collagen. It plays a role in initiating signal transduction within cartilage on load bearing ***(Wilusz et al., 2014).***

II) **Territorial Region:** Area around pericellular matrix; contains a basketlike network of type VI collagen microfibrils surrounds chondrocyte protecting it from mechanical stresses contribute to articular cartilage ability to withstand load ***(Guilak and Mow, 2000).***

III) **Interterritorial Region:** The largest region; contains abundant PG, randomly oriented bundles of large collagen fibril. It contributes to most of the biomechanical properties of the articular cartilage ***(Sophia Fox et al., 2009)***

The components of articular cartilage

I- Extracellular matrix components

i- Collagens

The most abundant proteins in the ECM about 60% of articular cartilage weight. It consists of 3 polypeptide chains (α-chains) wound into a triple helix called tropocollagen; Figure (4). The amino acids of the polypeptide chains are glycine and proline with hydroxyproline providing stability via hydrogen bonds along the length of the molecule ***(Gelse et al., 2003).***

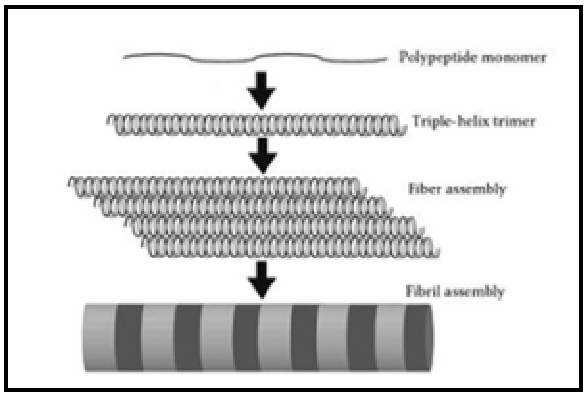

Figure (4): Collagen fiber assembly from polypeptide monomer to triple helix, to fiber and finally fibril assembly ***(Athanasiou et al., 2013a).***

Type II collagen represents 90% to 95% of the collagen in ECM and forms fibrils and fibers entangled with PG aggregates. It is composed of 3 identical α1(II) chains. Collagen types VI, IX, and XI, XII and XIV are also present but contribute only a minor proportion, they may have important structural and functional properties ***(Goldring, 2013).***

Collagens IX and XI are specific to cartilage, whereas collagens VI, XII, and XIV are widely distributed in other connective tissues ***(Aigner et al., 2007)***. Small amounts of collagen III are found in cartilage, and collagen VI may increase in OA cartilage. Collagen XII and XIV, are structurally related to type IX collagen and do not form fibrils by themselves but coaggregate with fibril forming collagens and modulate the packing of collagen fibers through domains projecting from their surfaces ***(Eyre et al., 2006).***

The collagen I triple helix is usually formed as a heterotrimer by two identical α 1(I) chains and one α 2(I)-chain.

It forms more than 90% of the organic mass of bone and is the major collagen of tendons, skin, ligaments, cornea, and many interstitial connective tissues with the exception of very few tissues such as hyaline cartilage, brain, and vitreous body ***(Gelse et al., 2003).***

ii- Proteoglycans

It forms 10–20% articular cartilage weight. It consists of a protein peptide core substituted with one or more glycosaminoglycan (GAG) chains (linear polysaccharide chains) ***(Darling et al., 2004).***

The GAGs are repeating, unbranched polysaccharide chains composed of repeating unit formed of six carbon sugars (hexose or hexuronic acid) linked to nitrogen containing a six carbon sugar (hexosamine). The common GAGs present in the articular cartilage are chondroitin sulphate (CS), keratin sulphate (KS), dermatan sulphate (DS), and hyaluronan (HA) where CS and KS are the most prevalent, the CS chains exist in different sulphated forms (sulphated at 4-O-hydroxyle in fetal cartilage and at 6-O- hydroxyle at adult cartilage), also the carboxyl (COO^-) and sulphate (SO_3^-) groups present on these attached GAGs produce a strong negative charge giving the ECM a net negative charge known as "fixed charge density" ***(Athanasiou et al., 2013a).***

There are two types of PGs in the articular cartilage which are large aggregating PGs (Aggrecan), and the small