Introduction

Tooth development starts at 5th week of human gestation and at 10th embryonic day of mouse development. Each tooth passes through four morphological stages: initiation, bud, cap, and bell stages, named according to the shape of enamel organ [1]. At late bell stage, odontoblasts secrete first layer of dentin starting dentinogenesis, dentin formation, and amelogenensis, enamel formation, through reciprocal induction.

Collagen is the major insoluble fibrous protein in the extracellular matrix and in connective tissue [2]. Type I collagen forms a fibrous three-dimensional network which builds up the dentin matrix. Compared to bone, the collagen matrix in dentin is more interwoven with numerous crossings of fibrils [3]. Excessive degradation of type I collagen is associated with a variety of human diseases such as RA, tumor metastasis, and atherosclerosis [4].

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by joint swelling, joint tenderness, and destruction of synovial joints, leading to severe disability and premature mortality [5]. RA is considered an autoimmune disease due to presence of auto-antibodies, such as rheumatoid factor (RF) and anti-citrullinated protein antibody (ACPA) (tested as anti-cyclic citrullinated peptide [anti-CCP]), which can precede the clinical manifestation of RA by many years [6].

Many women with RA are of childbearing age, which highlights the importance to study if there is any effect on tooth development of their children. To our best knowledge, this is the first study that evaluates the effect of rheumatoid arthritis rat mother on tooth development of their offspring.

Materials and methods

Animals

20 female Albino rats weighting (180-200 g) were used in the study and divided into 2 groups: control group (CG), and rheumatoid non-treated group (RhG).

Collagen preparation

Arthritis was induced successfully according to the protocol recommended by Chondrex Inc. Type II collagen (CII), isolated and purified from bovine articular cartilage (Chondrex, Inc.), dissolved overnight at 4°C in 0.01 M acetic acid at a concentration of 2 mg/ml. The solution was then emulsified in an equal volume of incomplete Freund’s adjuvant (IFA) (Sigma) in a drop-wise fashion with continuous stirring with electric homogenizer. The stability of the emulsion was tested by adding one drop of emulsion into a beaker of water. A stable emulsion remained, as a solid clump in water without dispersing, while the spreading onto the water surface indicated an unstable emulsion [7].

Histological and immunohistochemical analysis

Tongue specimens were fixed in 10% neutral buffered formalin for 24 hours then were trimmed and processed by standard paraffin-embedding methods. Sections were cut at 4 μm, deparaffinized, and then stained with: H&E & Immunohistochemical staining using monoclonal antibodies to collagen I (COL-1).

Results

Histological analysis

H&E stained coronal sections were examined of fetuses of CG and RhG for developing tooth germ of the 1st molar. In CG, at 1st day after birth tooth germ of developing 1st molar was at late bell stage with definite layer of dentin and at 10th day dentin and enamel matrix formation were almost completed. In RhG, at 1st day after birth tooth germ of developing 1st molar was at early bell stage; without dentin formation and at 10th day normal thickness of enamel matrix and dentin were formed. (Fig. 1 A&B) and (Fig. 2 A&B).

Immunohistochemical analysis

COL-1 was more brown intense in developing dentin and bone in CG than in bone in RhG at 1st day after birth. On the other hand, the colour intensity of COL-1 at 10th day in
both groups was nearly the same. (Fig. 3 A&B) and (Fig. 4 A&B).

**Discussion**

Systemic auto-immune diseases have higher pervelance in woman and mainly at child bearing age [8]. Many studies observed that autoimmune disease in women during pregnancy may be associated with an increased risk for learning disabilities in their sons. In this study, RA is one of systemic autoimmune disease and is strongly associated with oral health [9]. This is to say because immunological and pathological processes occurring in periodontitis and RA are nearly identical. Prospective studies on pregnant mothers suggested that maternal periodontal disease may cause preterm birth, low birth weight and may increase their offspring's risk of developing early and severe dental caries [10]. So this study was conducted to answer the question, whether RA diseased albino rat mothers has any effect on tooth development in their offsprings.

In the present study, histological examination of developing 1st molar tooth germ at 1st day in RhG showed stage of early bell rather than late bell stage that was found in CG. This delay in tooth development of group B, compared with that in group A, can be explained by findings of Scott [11] who demonstrated that pregnant women with connective tissue diseases, such as RA, are at risk for preterm birth and intra-uterine fetal growth restriction. As a contradict, many clinical experience and the reports of over 500 patients in clinical studies demonstrated that RA activity decreases for many women during pregnancy and their baby born healthy [12]. However, this may be due to either low disease activity or good choice of medication taken by mothers during pregnancy.

Histological findings of RhG at 10th day, in this study, showed normal development as in CG. This was in agreement with findings of a cohort study of Dutch women with RA and was accomplished by de Steenwinkel et al. [13] who demonstrated that active rheumatoid arthritis (RA) during pregnancy, without medication, and the presence of RF or anti–citrullinated protein antibodies (ACPAs) are associated with lower birth weight of the child followed by rapid postnatal catch-up in weight.

In the present study, Immunohistochemical analysis showed that brown colour intensity of COL-1 in RhG at 1st day was less than that of CG indicating that secreted collagen I was decreased in RhG. This result could be explained as the following: auto-antibodies against collagen type I that circulating in blood of high disease activity RA pregnant mothers were able to cross placenta and attack collagen type I in developing bone and dentin resulting in transient slow rate of formation and decreased amount of collagen in these tissues [14,15]. However, this was not the case at 10th day, where the colour intensity of COL-1 was the same in RhG and CG which may be also due to rapid catch up mechanism.

**Conclusion**

With the limitation of our study as it was the first study that investigate the effect of one of maternal autoimmune disease, RA, on tooth development, we can conclude that RA, under certain circumstances as absence of medication or high disease activity, leads to slow rate of development and decreased amount of collagen I production at first few days after birth and compensated by rapid compensatory mechanism and restoration of normal rate of development and growth at the following days.

*Figure 1: Photomicrograph of developing 1st molar at 1st day, CG (A) and RhG (B). (H&E stain, x 100)*
Figure 2: Photomicrograph of developing 1st molar at 10th day, CG (A) and RhG (B). (H&E stain, x 400)

Figure 3: Photomicrograph of developing 1st molar at 1st day, showing positive brown intense reaction of COL-1 in dentin and bone of CG (A) and less intense reaction in bone of RhG (B). (COL-1 stain, x 100)
Figure 4: Photomicrograph of developing 1st molar at 10th day, showing positive brown intense reaction of COL-1 in dentin and bone of both CG (A) and RhG (B). (COL-1 stain, x 100)

References