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## **The Effect of Pregnancy with Periodontitis on the Level of Metallomatrix Protinease- 9**

A research Submitted to the department of Periodontics  
Dentistry in the Faculty of Dentistry , University of Babylon  
as a part of requirement of bachelor degree ( B.D.S. )

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بسم الله الرحمن الرحيم

يرفع الله الذين آمنوا منكم  
والذين أوتوا العلم درجات والله  
بما تعملون خبير

صدق الله العظيم

سورة المجادلة . آية 11

## **Certification**

**I certify that this research has been prepared under my supervision at the college of Dentistry , University of Babylon .**

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**Lect. Assist. Douaa Neama Mseer**

## الإهداء :

إلى أصحاب الكلمات التي سارت بي نحو النجاح ,

إلى من ساندوا خطاي المتعثرة

إلى من لا يضاهيهما أحد في الكون ,

إليكما أهدي هذا الجهد، وهذا البحث، فقد كنتما على الدوام ملهمي، فعلى خطاكما أسير، وبعلمكما أقتدي، أمي وأبي،  
أشركما الشكر الجزيل على ما قدّمتماه لي طوال فترة دراستي، وإنجازي لهذا البحث .

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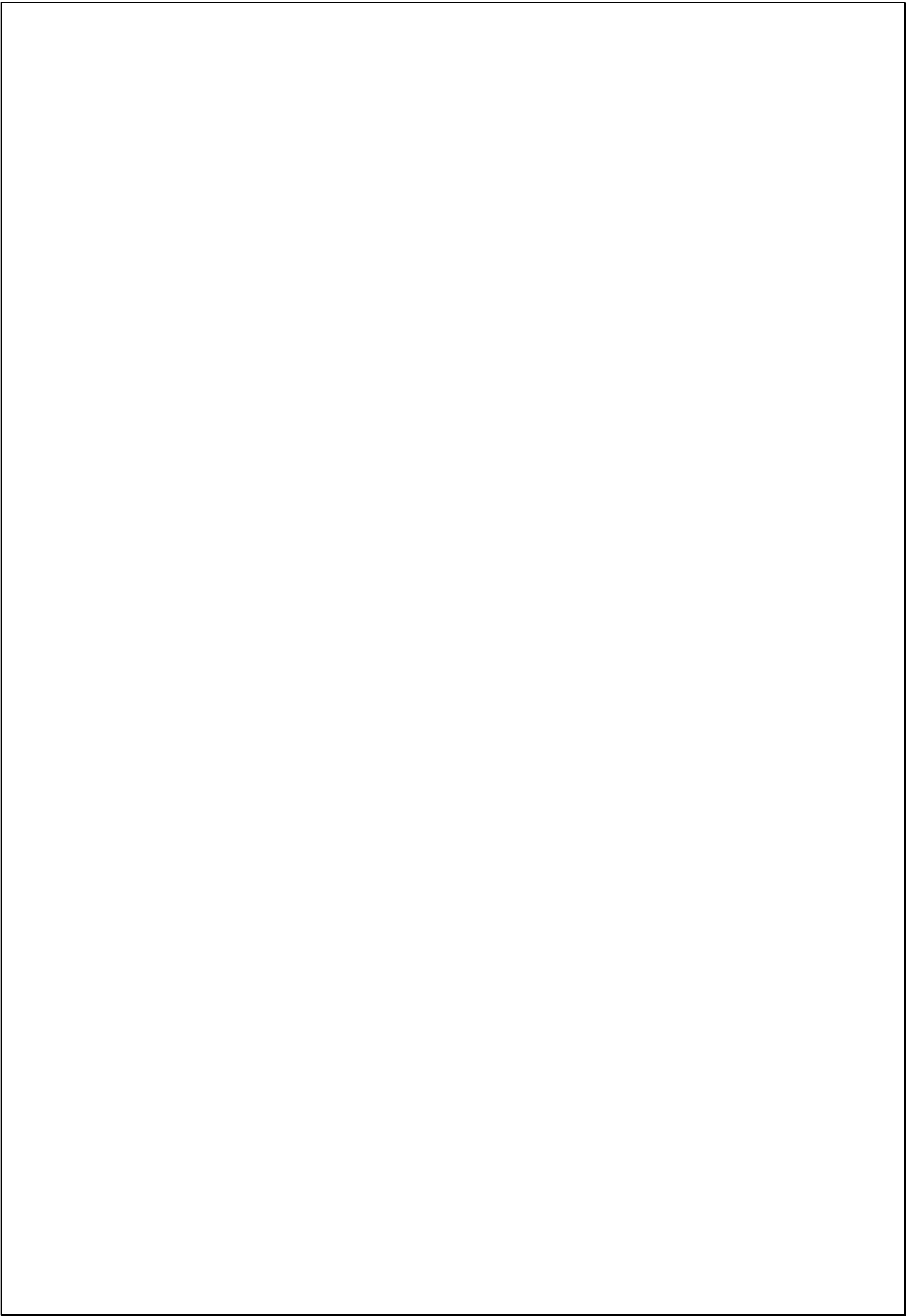
## **The Students.**

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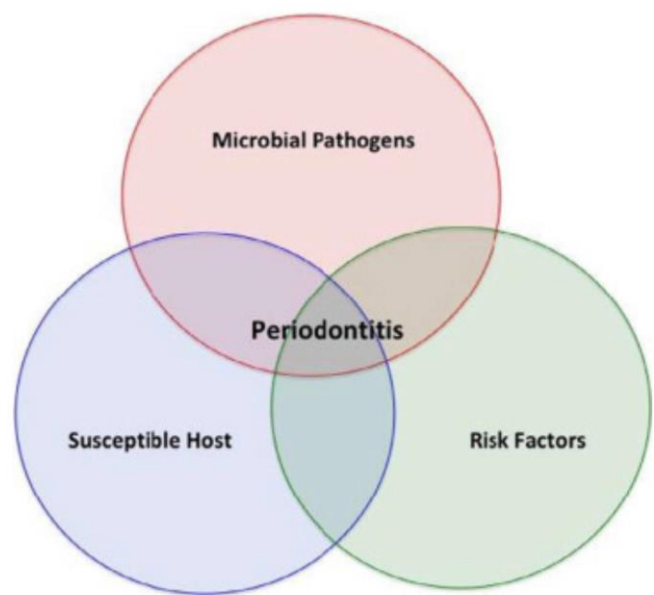
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# **The effect of pregnant women with periodontitis on the level of matrix metalloproteinases MMP-9**

## **Introduction**

Periodontitis is a chronic multifactorial inflammatory disease associated with the accumulation of dental plaque (which will be referred to as dental biofilm/biofilm), and characterised by progressive destruction of the teeth-supporting apparatus, including the periodontal ligament and alveolar bone. (1,2) The disease involves complex dynamic interactions among specific bacterial pathogens, destructive host immune responses, and environmental factors such as smoking.(1,3) The common features of periodontitis include gingival inflammation, clinical attachment loss, radiographic evidence of alveolar bone loss, sites with deep probing depths, mobility, bleeding upon probing and pathologic migration.(2,4,5)



Periodontal disease shares risk factors with cardiovascular diseases and other systemic inflammatory diseases and also pregnancy, Nearly 60 to 75% of pregnant women have gingivitis and periodontitis , an early stage of periodontal disease that occurs when the gums become red and swollen from inflammation that may be aggravated by changing hormones during pregnancy(17). If periodontitis is not treated, the bone that supports the teeth can be lost, and the gums can become infected. Teeth with little bone support can become loose and may eventually have to be extracted. Periodontitis has also been associated with poor pregnancy outcomes.(18)



Matrix metalloproteinases (MMPs) are key proteases for tissue turnover, and their activity is regulated by endogenous inhibitors, the tissue inhibitors of MMPs (TIMPs). Periodontal disease is an inflammatory process with increased MMP activity in the periodontal tissues, resulting in a massive loss of collagen in the periodontal structures even at the very early stages of periodontal disease. MMP-2, MMP-3, MMP-8, and MMP-9 are increased in inflamed gingival tissue (6).

Increased MMP-9 have been described in gingival crevicular fluid (GCF) during the active stages of periodontal disease (7,8,9). Elevated MMPs have been associated with increased inflammation and loss of tooth-supporting tissue present in periodontal disease, while periodontal treatment decreases inflammation and lowers MMP-9 levels,

As for bone resorption, MMP-9 is likely the most important proteinase involved in this process as osteoclasts express this enzyme at a tremendously high level

MMP-9 is increased in gingival crevicular fluid and salivary fluid during pregnancy in patient with periodontal disease .

This study evidenced that pregnant women with periodontitis presented elevated MMP-9 concentrations in saliva compared to healthy subjects. So its concentrations in saliva can be considered as biochemical indicators of severity of periodontitis

The aim of this study was to identify whether the levels of matrix metalloproteinase-9 are increased due to pregnancy

## **Methods and Materials**

The saliva samples were obtained from 40 participants in to two groups, 20 pregnant women with periodontitis with age range (31-43) and 20 control groups with age range (23-50) were

included in present study. Specified by measuring periodontal parameters including, pocket depth, plaque index and Bop (bleeding on probing). Exclusion criteria include: Patients with systemic diseases. And Patients with previous periodontal treatments or who take . .medication

### **Saliva collection and Storage:**

Non-stimulated clean saliva was collected in sterile laboratory cup and stored in a cool box containing ice bags to maintain its viability till it taken to the laboratory examination, then each sample was centrifuged at (3000 rpm) for 10 min. to make separation for the undesired free salivary particles. After that the clear salivary solution was aspirated by using (1µl) micropipette tips and save it in (1ml) sterile Eppendorf tube for freezing under (-20°C) to make the final Immunological examination study by the enzyme-linked immunosorbent assay (ELISA) tests.

### **Quantitative determination of MMP-9**

Quantitative determination of MMP-9 in saliva of patients and control group using Enzyme-linked Immunosorbent Assays (ELISA) as in manufacture instructions Sandwich (BT LAB)

### **Statistical analysis**

All statistical analysis are calculated by using SPSS version 26 software Continuous data were expressed as means±SD, median, minimum and maximum Categorical variables are presented as absolute numbers and percentages. Kolmogorov-Smirnov test is used to determine the normality distribution of data. Comparison of continuous variables was analyzed using Independent Samples T Test for normally distributed data, and Mann Whitney U Test for not normally distributed data. Categorical variables were analyzed with Pearson's chi-squared test and Fisher's exact test. P value of less than 0.05ng/L was considered significant.

### **Exclusion criteria**

Smokers

any patient had history of other chronic systemic diseases with known associations with , PD as cardiovascular diseases and diabetes mellitus were not included in the study antibiotics or anti-inflammatory agents within the previous 3 months or had periodontal .treatment in the past 3 months

### **Inclusion criteria**

- Healthy patients
- Pregnant women with periodontitis

ELISA machine



### **Results**

Saliva samples were obtained from 20 women patients with periodontitis and from 20 control people. Demographic parameter and clinical descriptive data in both groups showed in table 1 and table 2. (Table 1, Table 2, Figure 1).

The mean age for periodontitis patients were  $(31.25 \pm 6.84)$

And the mean age for control (healthy) group was  $(25.05 \pm 6.653)$ .

\*The results of current study revealed that higher significant differences in periodontal parameter (plaque index, pocket depth, and bleeding on probing) between pregnant women with periodontitis and control group ( $<0.001$  and  $0.005$  respectively).

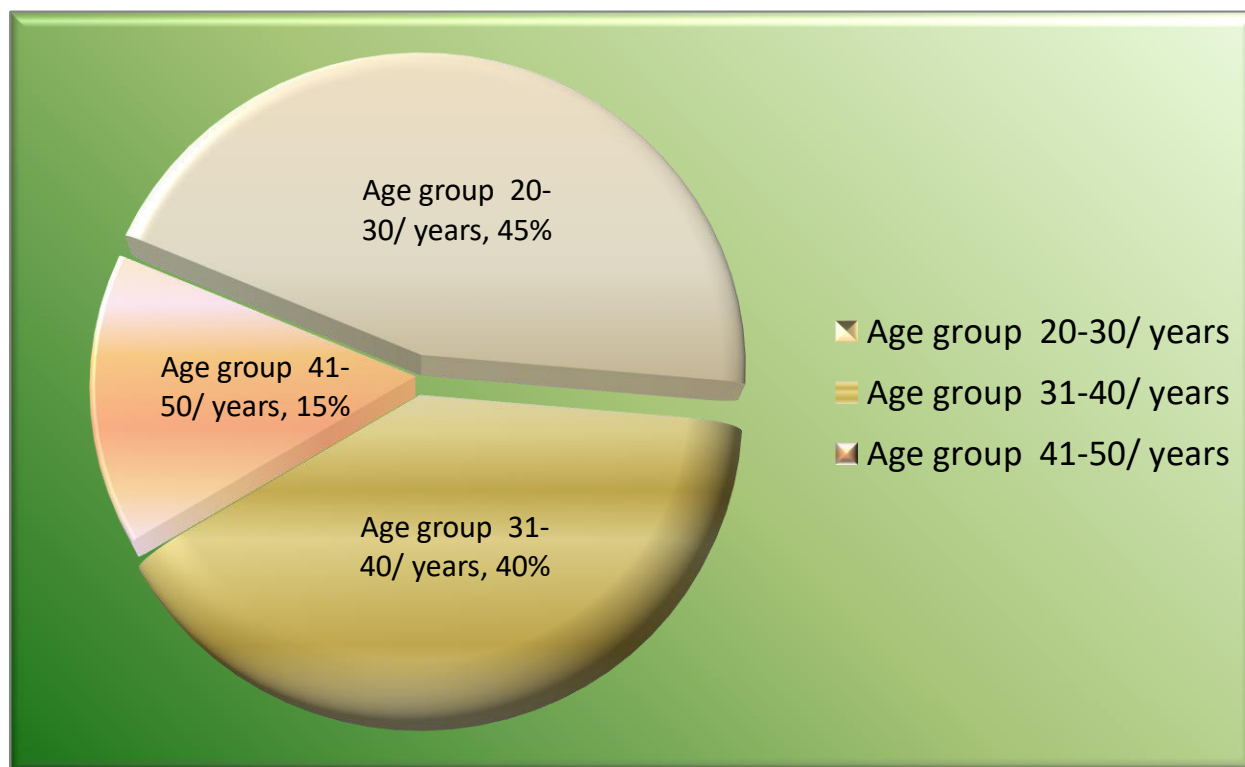
Table 1: General demographic and clinical descriptive data in both control and pregnancy groups .

parameters	pregnancy with periodontitis (n = 20)				Control group (n = 20)			
	mean±SD	Median	Minimum	Maximum	mean±SD	Median	Minimum	Maximum
Age	31.25±6.84	31	21	43	25.05±6.653	23	20	50
Plaque index	1.148±0.513	1.305	0.304	1.86	0.476±0.24	0.47	0.132	0.8
pocket depth / mm	2.687±1.867	2.398	0.04	5.36	0.604±0.59	0.518	0	1.56
Bleeding on Probing	0.325±0.425	0.242	0	1.607	0.05±0.088	0.012	0	0.33
MMP-9 concentration	1827.2±718.7	1887.8	750.5	2646.9	1271.3±594.6	1093.1	487.7	2564.6

Table 2: Comparison demographic data between pregnancy with control groups and their significant difference .

Parameters	pregnancy group with periodontitis (n = 20)		Control group (n = 20)	*P value
Age group / years old	20-30	9(45%)	18(90%)	0.009
	31-40	8(40%)	1(5%)	
	41-50	3(15%)	1(5%)	

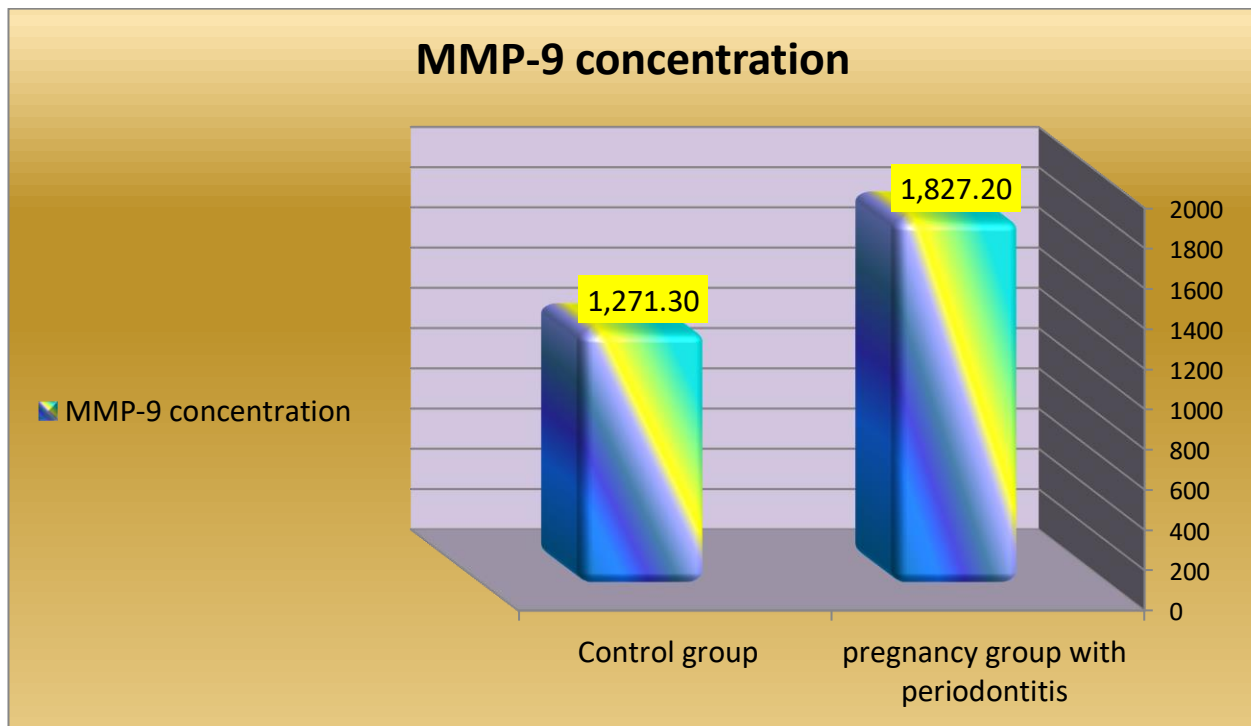
\*Fisher's exact test



**Figure 1** distribution of pregnancy with periodontitis disease according to age group

Table 3: significant difference of clinical and biochemical data between pregnancy and control groups .

Parameters	pregnancy group with periodontitis (n = 20)	Control group (n = 20)	P value
Age ( years )	31.25±6.84	25.05±6.653	<b>*0.006</b>
Plaque index	1.148±0.513	0.476±0.24	<b>*&lt; 0.001</b>
pocket depth / mm	2.687±1.867	0.604±0.59	<b>*&lt; 0.001</b>
Bleeding on Probing	0.325±0.425	0.05±0.088	<b>**0.005</b>
MMP-9 concentration	1827.2±718.7	1271.3±594.6	<b>**0.024</b>
*Independent Samples T Test			
**Mann-Whitney test			



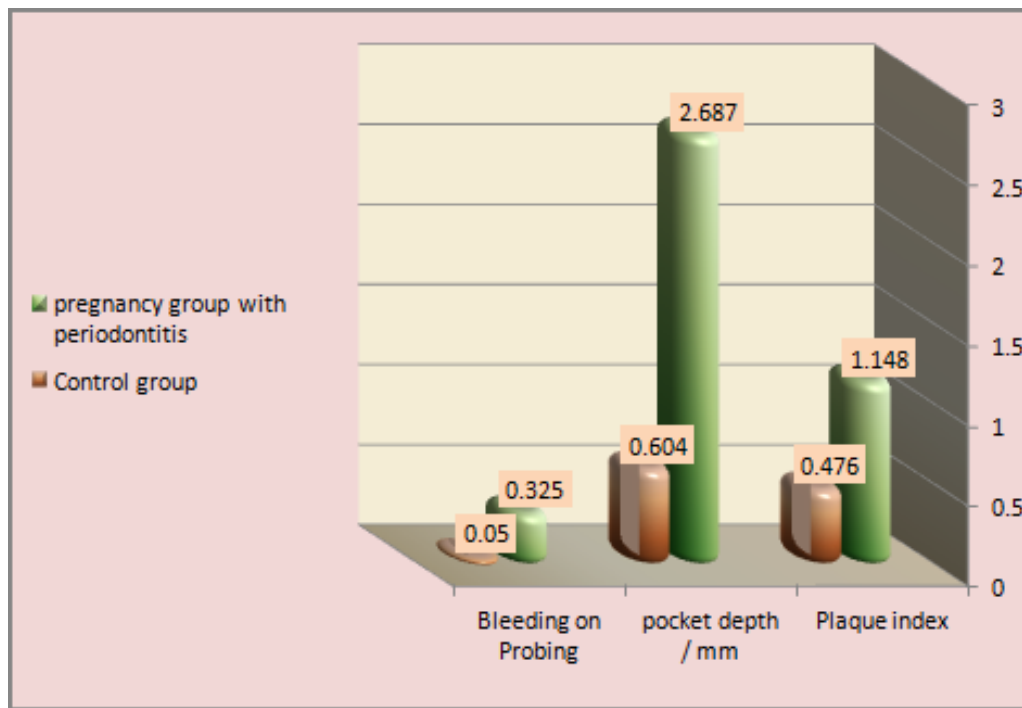
**Figure 2 Mean count of distribution of MMP-9 concentration in comparison between pregnancy and control groups**

The results revealed the saliva concentration of MMP9 is as follow:

The mean concentration for pregnant women with periodontitis is  $(1827.2 \pm 718.7)$ , And for control group is  $(1271.3 \pm 594.6)$ .

\*it was observed that MMP9 concentration of pregnant women with periodontitis show a significant increase compared to control group, The result showed significant difference, increase in the concentration of MMP in patient than control, according to (figure 2).

\*The test group demonstrated mean concentration of  $(17 \pm 5 \text{ D mg/ml})$  compared to mean concentration of  $(12 \pm 5 \text{ D mg/ml})$  ( $p < 0.001$ ) in the control group.



**Figure 3 Mean count of Plaque index, pocket depth, and Bleeding on Probing groups in comparison between pregnancy and control people**

The final differences between pregnant women with periodontitis and control group in periodontal parameters and MMP9 level ,according to (figure3).

## Discussion

Periodontitis is an inflammatory disease that is modulated by several factors, among which members of the MMP family play an important role in the degradation of the extracellular matrix and the destruction of periodontal tissue. Due to their particular physiopathological pattern, MMPs are strong predictors for periodontitis.

There is association between specialy periodontids and pregnancy .Periodontits during pregnancy starts by dental plaque and is increased by the action of pregnancy hormones. In order to study the effect of Periodontits on adverse pregnancy outcome.

In our current study, in table 1 the result of mmp9 of pregnancy with periodontits (1827.2\_+718.7).In the research of Sechenov University Ethics Committee (No. 10-17, November 16, 2017) the results were (1391\_+599) which approximately agree with our results while our results disagree with research of Reproductive biology and encrinology of BMC journal (15 Jan 2013) the results were (165) , the differences in the results maybe due to numbers of samples ( he use 126 samples and ours was 20 samples) or the time of taking samples in which trimester or maybe due to incorrect saving of samples during collecting or faulty reading to parameters.

This study evidenced that pregnant women with periodontitis presented elevated MMP-9 concentrations in saliva compared to healthy subjects. So its concentrations in saliva can be considered as biochemical indicators of severity of periodontitis



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