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Relationship between Gingival Inflammation, Neutrophils Count in both Saliva and Blood in different stages of Pregnant Iraqi Women

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Abstract

Neutrophil, plays an important role in innate immune response, that phagocyte bacteria, will burst of oxygen consumption (respiratory burst), and produce reactive oxygen type as bactericidal or by produce of lysis enzymes (non oxygen dependent). Phagocytes are the important factors that play a role in acute inflammatory response due to their ability to destroy various pathogens efficiently. Gingival changes in pregnancy have been attributed to changes in sub gingival biofilm related to hormonal variation.

Aim of the study: To evaluate the neutrophils count in both saliva and blood, also determined the phagocytic activity of neutrophil in pregnant at different stages and non-pregnant women the study the association of neutrophile changes with severity of some oral disease including gingivitis and plaque.

Material and methods: twenty four pregnant women as study group and fourteen non pregnant women as control group were included in this study. The pregnant women were following up for three groups according to pregnant stages .Plaque and gingival indicates. Saliva and blood samples from pregnant women at different stages and control group (non pregnant women) were obtained and neutrophils total number was determine by using Neubauer chamber so the neutrophils number were carried out for all subjects . The phagocytic activities of neutrophils were examined after challenged the neutrophils with *candida albicans* that had been opsonized.

Conclusion: salivary and blood neutrophils counting and phagocytic function are significantly increase in women at third trimester and positively associated with the severity of plaque and gingivitis.

Key wards: neutrophil, plaque, gingivitis, pregnant, phagocytosis.

Introduction

Neutrophils or polymorph nuclear leukocytes plays a role as one of the front line of body's defense against infection. These cells use bactericidal pathways that dependent or independent oxygen as a weapon to eliminate infectious agents [1]. Oxygen-dependent mechanism involves production of bactericidal reactive oxygen compounds. The mechanism of oxygen-independent involves chemotaxis, phagocytosis, degranulation, and the release of lyses enzymes and bactericidal peptides. [2]

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In response to bacterial pathogens entrance, neutrophils will move into the infected tissue, then activate to form a reactive oxygen compounds. This event called respiratory burst involving the NADPH oxidase activation. At the respiratory burst, there was a rapid uptake of molecular oxygen and transformation into reactive oxygen compounds, which on one side is a representation of the host defense mechanisms in the inflammatory site, on the other hand the possibility of damage close to healthy tissue. It is important that relevant reactive oxygen compounds physiological concentrations able to modulate the redox -sensitive signaling cascade and improve immunological cellular function [3]. Reactive oxygen compounds can trigger oxidative damage to macromolecules, leading to lipid peroxidation, amino acid chains oxidation forming protein fragmentation, DNA strands ruptured. [4].

Periodontal disease is defined as an inflammatory reaction to microbial infection associated with dental plaque results in tissue loss. With plaque accumulation and development of clinical inflammation there is an increase in the number of leuckocytes present in the lesion. [5]. during pregnancy, about half of all women (60-70) % experience a condition called pregnancy gingivitis. It is caused by an increase in the hormone levels, which can exaggerate a women's response to dental plaque in the mouth. This extra plaque may cause swelling, bleeding, redness and or tenderness in the gums .Changes in the gums is most noticeable from the second month of pregnancy, reaching a maximum at the eight month. [6] To control the amount of plaque in your mouth and to prevent gingivitis, brush your teeth regularly at least twice a day for two minutes; and floss your teeth every day. These actions help reduce the bacteria that can lead to pregnancy gingivitis. [7] The present study was performed to study the neutrophil count and phagocytosis activity of PMN in pregnant women at different stages compared to non pregnant in both saliva and blood sample also, to performed the association between the neutrophil count, phagocytic activity with severity of disease in pregnant women.

Material and Methods

The total sample consisted of forty six(46) pregnant women, they were collected from outpatient of Al karama hospital and privet clinic they were divided into twenty eight(28) pregnant women as study group twenty four(24) from these women were diagnostic with gingivitis while four(4) women from this study group without gingivitis. Also thirty (30) non pregnant women randomly selected women from which eighteen women selected as control group. Eighteen women (non pregnant women) control group were asked to record the date of their menstrual period so as to standardize the time of examination. The groups were designated as group I (first trimester), group II (second trimester), group III (third trimester) and group IV (control group) .patients selected for this study should be following these criteria: No history of any systemic disease or chronic diseases, no history of antibiotic drugs therapy during the past 6 weeks, no history of dental oral prophylaxis during the past six months and no history of oral contraceptive use for control groups.

Three to five ml of saliva and blood as a sample were collected from both study and control group, then started for isolation of salivary and blood neutrophils cells by modified method

by lukac et. al [8] .In which from each group 10 ml, of sterile NaCl solution without swallowing for four minutes and expectorated into a sterile beaker. This procedure was further repeated four times, then, centrifuge at 4500 for 10 minutes then supernatant was discharged and the pellet suspended in 2 ml of RPMI 1640 medium containing 10% of fetal calf serum (10% FCS RPMI), then vortexes to avoid aggregation of neutrophils and filtered the suspensions by nylon filters, then washed by 10%FCS RPMI medium. In addition the heparinized whole blood was obtained and purified, washed then salivary and blood neutrophils were identified by staining with Turk's solution. The viability of salivary and blood neutrophils was analyzed and counted by Neubauer chamber [9] .

The phagocytosis of neutrophils were detected after purified of neutrophil cells and challenged with viable blastoconidia of *candida albicans* that had been opsonized with human serum and after 30 and 120 min, percentage of salivary and blood neutrophils phagocytosis candida albicans assessed.

For each patient, mouth plaque Index (Silness &Loe, 1964) [10] and gingival index (Silness &Loe, 1963)[11] were recorded at all surfaces per tooth (buccal, lingual, mesial and distal) surfaces.

Statistical analysis:

The collected data was performed using the statistical SPSS-19, ANOVA test of independent means was used applied for the qualitative data for testing the significance of difference between mean and proportion respectively. P –value equal or less than 0.05 was considered as the level of statistical significance. Also using Dunnetti test for multiple comparison as well as using correlation coefficient test.

Results

Table -1- shows Descriptive statistics of gingival and plaque index of pregnant and non pregnant women control group. The study groups were classified into three groups according to the trimester of pregnancy. The highest mean value of studied variables for gingival and plaque index was reported in third trimester of experimental groups mean (2.9±0.3 SD, 2.6±0.3 SD respectively), also the mean value of gingival and plaque index was increased with progression of pregnancy, where the mean value in the third trimester is higher than first and second trimester as seen in figure-1-.

Table-2- showed the difference in mean of gingival and plaque index among studied group, a high statistical significant difference among groups regarding gingival and plaque index (p value≤0.05), where the highest mean value (2.9) of gingival and plaque index(2.6) was reported in third trimester and it was higher than other groups. On multiple comparisons by using Dunnetti test, a high statistical significant difference was also noted as seen in table -3-.

Table-4-showed the difference in mean of salivary neutrophil among studied groups, a high statistical significant difference among studied groups (p value≤0.05) in which the mean value of salivary neutrophil count (3.1) in the third trimester higher than other experimental groups (first and second trimester)mean value (1.2,2.1)respectively, also more than control group (0.3), as seen in figure-2-.On multiple comparison by using Dunetti test significant statistical difference was also noted as seen in table 5.

Table-6-showed mean of blood neutrophil among studied group, a high statistical significant difference among

studied groups (p value≤0.05), the mean value of neutrophil count in blood (333.3) in the third trimester is higher than other experimental groups (first and second trimester groups) also more than control group as seen in figure-3-.On multiple comparisons a statistical significant difference also noted between groups (except first trimester group and control) as seen in table-7-.

Table-8- showed the difference in mean of phagocytic activity of studied groups, statistical analysis revealed that there was a significant elevation of phagocytic activity count in third trimester (38.2) compared to other groups (29.2and19.0) second and first trimester respectively as seen in figure-4. (p value≤0.05).Statistical significant difference also reported on multiple comparisons as seen in table-9-.

Discussion

The idea of using saliva in diagnostics was made in the second half of the 20th century. Its main advantage is easy and noninvasive sample taking compared to peripheral blood. Periodontal tissue destruction is governed by various host factors, such as nutrition, hormones, and immune mechanisms. Neutrophils or poly morphonuclear leukocytes play a critical role as a part of the innate immune response as a first line of defense against these invading microbes [12].

The presence neutrophils in the oral cavity have attracted interest for many years. In healthy and edentulous patients salivary leukocytes levels were lowest in comparison with patient with gingivitis.[13]In the healthy periodontium of human PMNs have been demonstrated migrating towards or residing within the sulcular and junction epithelium and within underlying connective tissue[14].In the

present study was verified by positive correlation seen between gingival index and neutrophil counts, this could be attributed to increase surface area of ulcerated epithelium and increase the migration of neutrophils through the ulcerated epithelium[15,16]in this study there is positive correlation between increase of pl scores and development of clinical inflammation and increase in the number of neutrophils present in the lesion[17,18]

Changes in female sex hormone levels during pregnancy are related to the enhanced susceptibility to gingival inflammation. This phenomenon, also named pregnancy-related gingivitis, typically occurs without a clear association with the amount of dental, and develops more severe forms, in comparison to plaque–induced gingivitis in non-pregnant develops more severe forms, in comparison to plaque–induced gingivitis in non-pregnant women [19]. The most common form of gingival diseases is plaque-induced gingivitis. According to the results of the Health 2000 Survey, 64% of Finnish women aged 30-34 years had gingivitis [20]

Several investigators have shown that hormonal imbalance seen during pregnancy can adversely affect the gingival tissues, which usually manifest in the form of an exaggerated inflammation. [21]. There are no studies that have been correlated the neutrophils in saliva of pregnant and non-pregnant women and their periodontal health status.

Conclusion

It has been conclude that neutrophil count and phagocytic activity of PMN cell had higher in blood and saliva of pregnant women than non-pregnant and significantly increase with the severity of gingivitis and the development of pregnancy.

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Table-1- Descriptive statistics of gingival and plaque index of pregnant at different pregnant stages and non pregnant women.

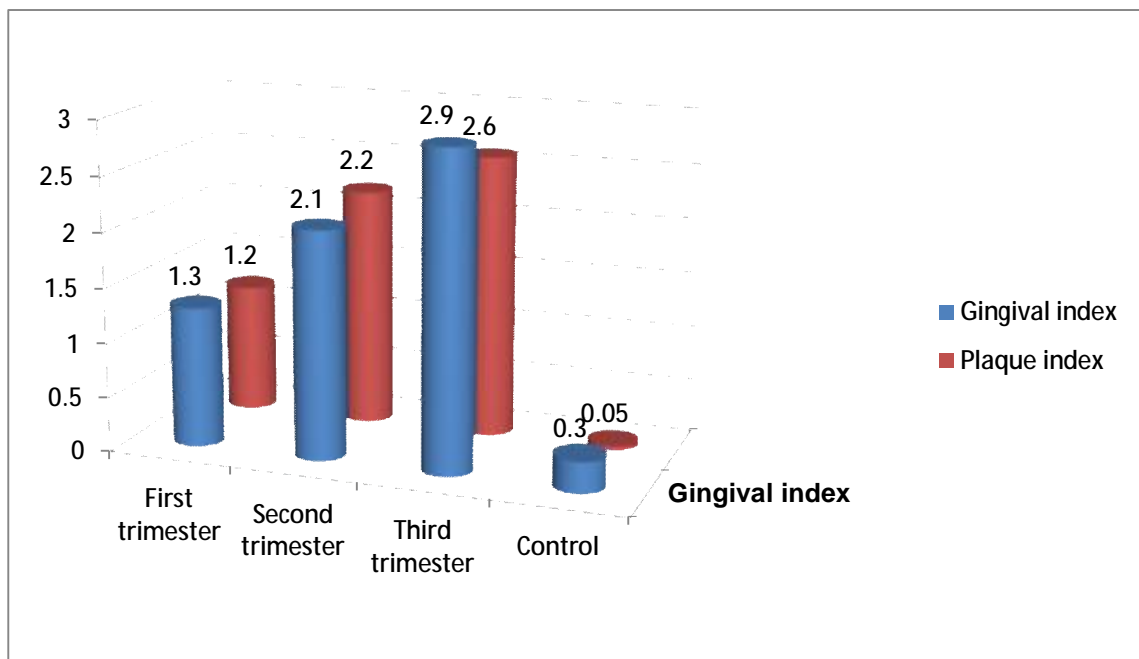| Std. Deviation	Maximum	Minimum	Mean	N	Study groups and variables	
0.1	1.6	1.1	1.3	24	Gingival index	First tri.
0.08	1.3	1.1	1.2	24	Plaque index	
0.3	2.8	1.6	2.1	24	Gingival index	Second tri.
0.2	2.5	2.0	2.2	24	Plaque index	
0.3	3.0	2.4	2.9	24	Gingival index	Third tri.
0.3	3.0	2.2	2.6	24	Plaque index	
0.2	0.8	0.0	0.3	14	Gingival index	Control
0.07	0.2	0.0	0.05	14	Plaque index	

Figure-1-Mean of gingival and plaque index for pregnant and non pregnant women.

Table-2-The difference in mean of gingival and plaque index among studied groups.

P- value (Anova)	95% Confidence Interval for Mean		Std. Deviation	Mean	N	Groups	Variables
	Upper Bound	Lower Bound					
0.001	1.3	1.2	0.1	1.3	24	First	Gingival index
	2.3	2.0	0.03	2.1	24	Second	
	3.1	2.9	0.3	2.9	24	Third	
	0.4	.1	0.2	0.3	14	Control	
0.001	1.2	1.1	0.08	1.1	24	First	Plaque index
	2.3	2.1	0.2	2.2	24	Second	
	2.8	2.4	0.3	2.6	24	Third	
	0.09	.006	0.07	0.05	14	Control	

Table-3-Mmultiple comparisons by Dennett- test (2 sided) of studied groups regarding gingival and plaque index.

95% Confidence Interval		Sig.	Mean Differenc e (I-J)	control group (pregnant without lesions)	experimental groups (pregnant with oral lesions)	Dependent Variable
Upper Bound	Lower Bound					
1.205	.768	0.001	0.9863*	Control	First trimester	Gingival index
2.038	1.601	0.001	1.8196*	Control	Second trimester	
2.913	2.476	0.001	2.6946*	Control	Third trimester	
1.295	.963	0.001	1.1292*	Control	First trimester	Plaque index
2.349	2.017	0.001	2.1833*	Control	Second trimester	
3.120	2.788	0.001	2.9542*	Control	Third trimester	

Table-4- The difference in mean of salivary neutrophil among studied groups.

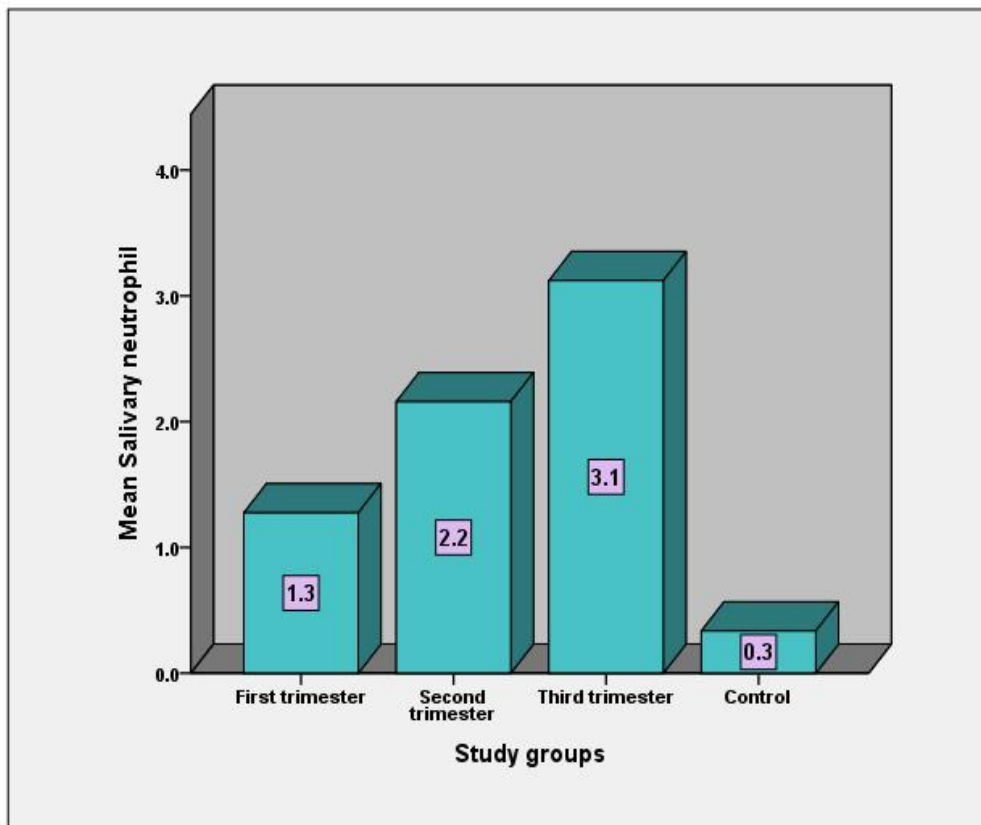| P- value	95% Confidence Interval for Mean		Std. Deviation	Mean	N	Groups
	Upper Bound	Lower Bound				
0.001	1.3	1.2	0.1	1.2	24	First trimester
	2.2	2.0	0.2	2.1	24	Second trimester
	3.2	2.9	0.2	3.1	24	Third trimester
	0.4	0.2	0.2	0.3	14	Control

Figure-2- Mean of salivary neutrophil for study groups

Table-5-Multiple comparisons of studied groups

95% Confidence Interval		Sig.	Mean Difference (I-J)	Control(pregnant free of disease)	Experimental groups (pregnant with diseases)
Upper Bound	Lower Bound				
1.129	0.750	0.001	0.9393[*]	Control	**First trimester**
2.012	1.633	0.001	1.8226[*]	Control	**Second trimester**
2.974	2.596	0.001	2.7851[*]	Control	**Third trimester**

Table-6- The difference in mean of blood neutrophil$\times 10^{5}$ among studied groups

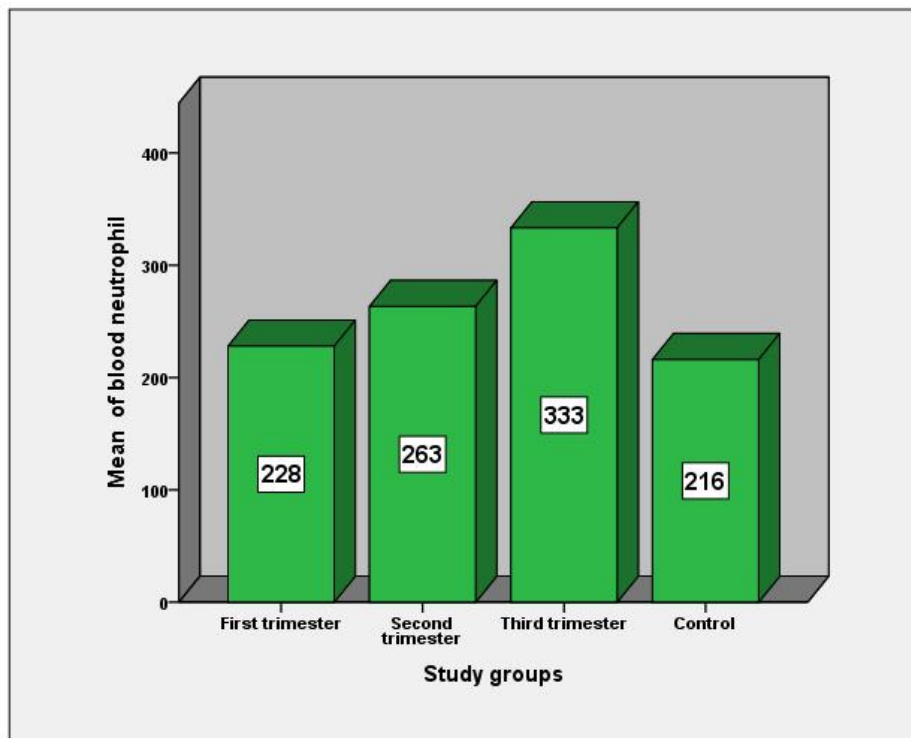| P-value	95% Confidence Interval for Mean		Std. Deviation	Mean	N	Groups
	Upper Bound	Lower Bound				
	233.2	222.6	12.5	227.9	24	**First trimester**
	269.8	256.7	15.5	263.3	24	**Second trimester**
0.001	345.0	321.6	27.7	333.3	24	**Third trimester**
	222.2	209.8	10.7	216.0	14	**Control**

Figure-3- Mean of blood neutrophil for studied groups.

Table-7-Multiple comparisons of studied groups

95% Confidence Interval		Sig.	Mean Difference (I-J)	Control	Experimental
Upper Bound	Lower Bound				
26.62	-2.93-	0.139	11.845	Control	**First trimester**
62.03	32.49	0.001	47.262*	Control	**Second trimester**
132.03	102.49	0.001	117.262*	Control	**Third trimester**

Table-8- The difference in mean of Phagocytic activity of studied groups.

P- value	95% Confidence Interval for Mean		Std. Deviation	Mean	N	Preg .stages
	Upper Bound	Lower Bound				
0.001	20.0	17.9	2.5	19.0	24	**First trimester**
	30.4	28.1	2.7	29.2	24	**Second trimester**
	40.0	36.3	4.3	38.2	24	**Third trimester**
	15.5	14.3	1.0	14.9	14	**Control**
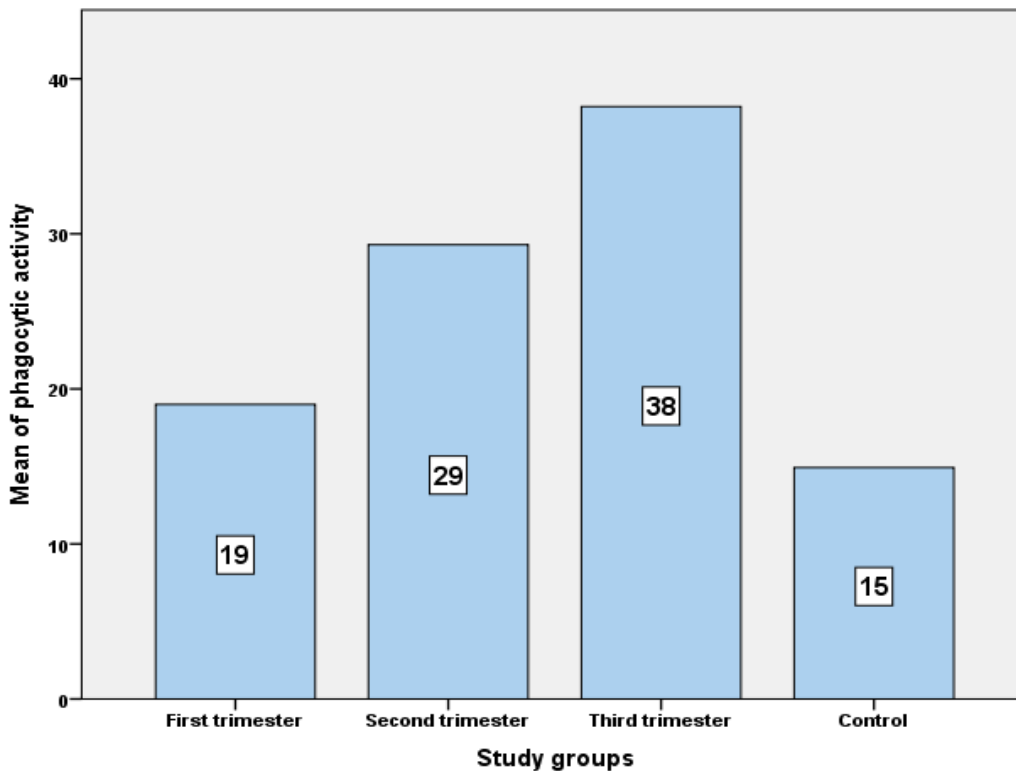
Figure-4- Mean of phagocytic activity for study groups

Table-9-Multiple comparisons of studied groups

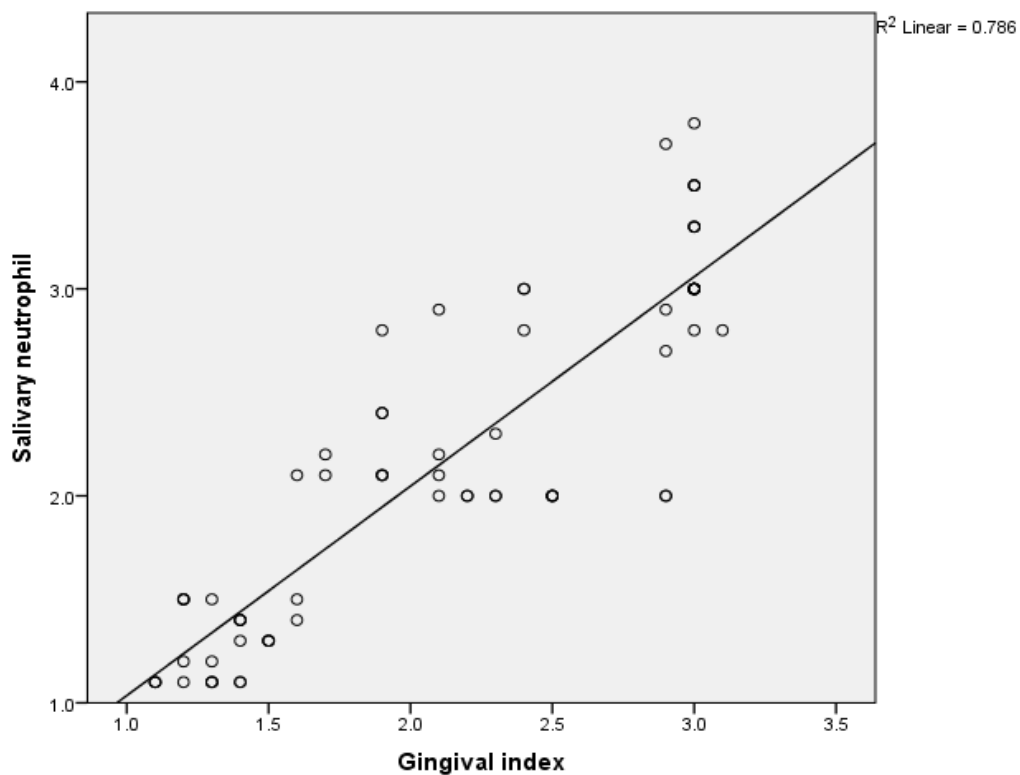| 95% Confidence Interval		Sig.	Mean Difference (I-J)	Control	Experimental groups
Upper Bound	Lower Bound				
6.53	1.61	0.001	4.071*	Control	**First trimester**
16.82	11.90	0.001	14.363*	Control	**Second trimester**
25.74	20.82	0.001	23.280*	Control	**Third trimester**

Figure-5-Relationship between salivary neutrophil and gingival index over three trimesters of pregnancy in experimental groups.
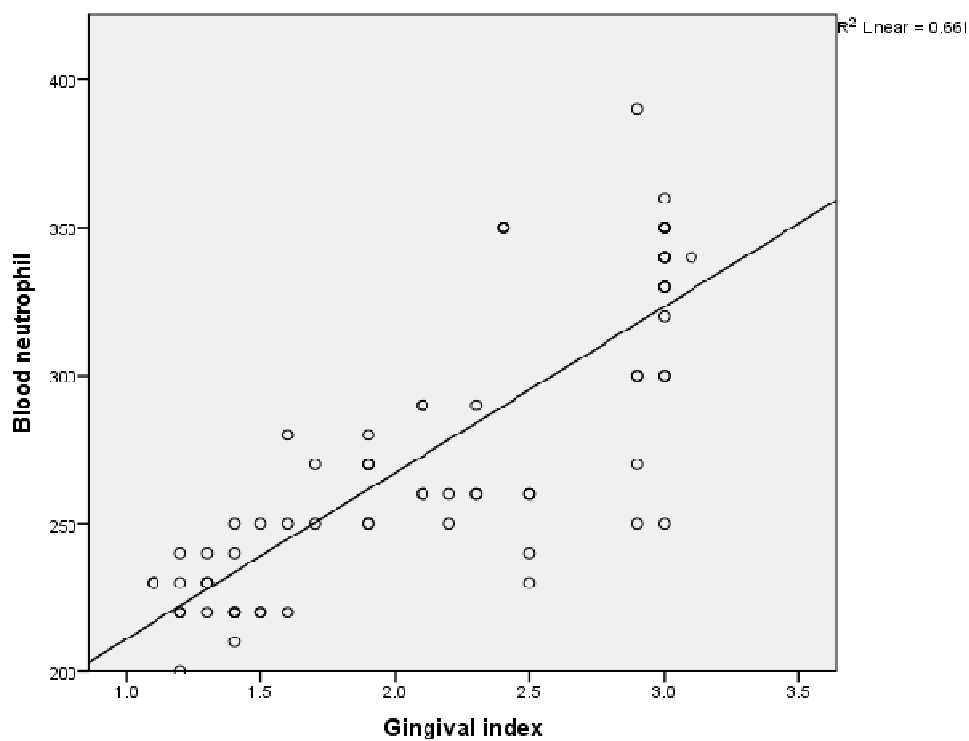

Figure-6-relationship between gingival index and blood neutrophil over three trimesters of pregnancy in experimental group.
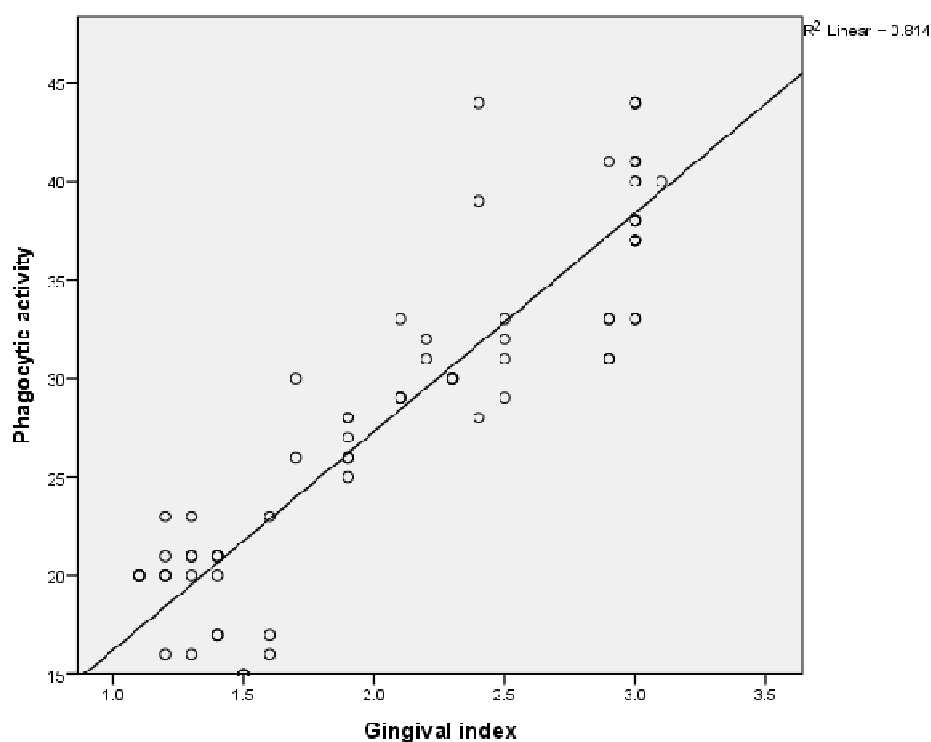
Figure-7-Relationship between gingival index and phagocytic activity over three trimesters of pregnancy in experimental group.
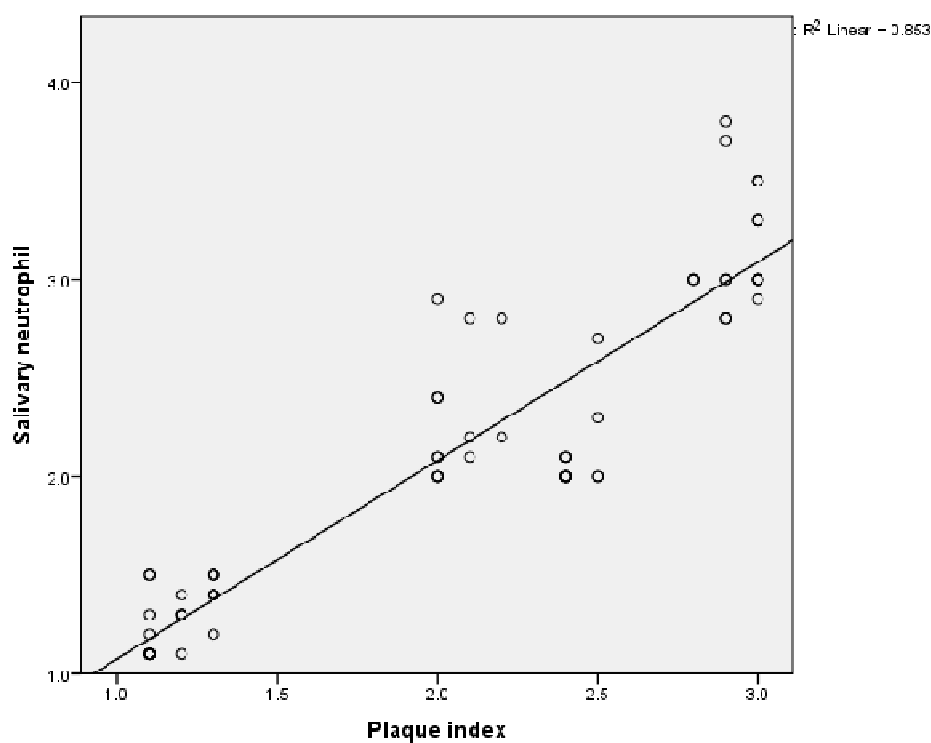

Figure-8-relationship between plaque index and salivary neutrophil over three trimesters of pregnancy in experimental group.
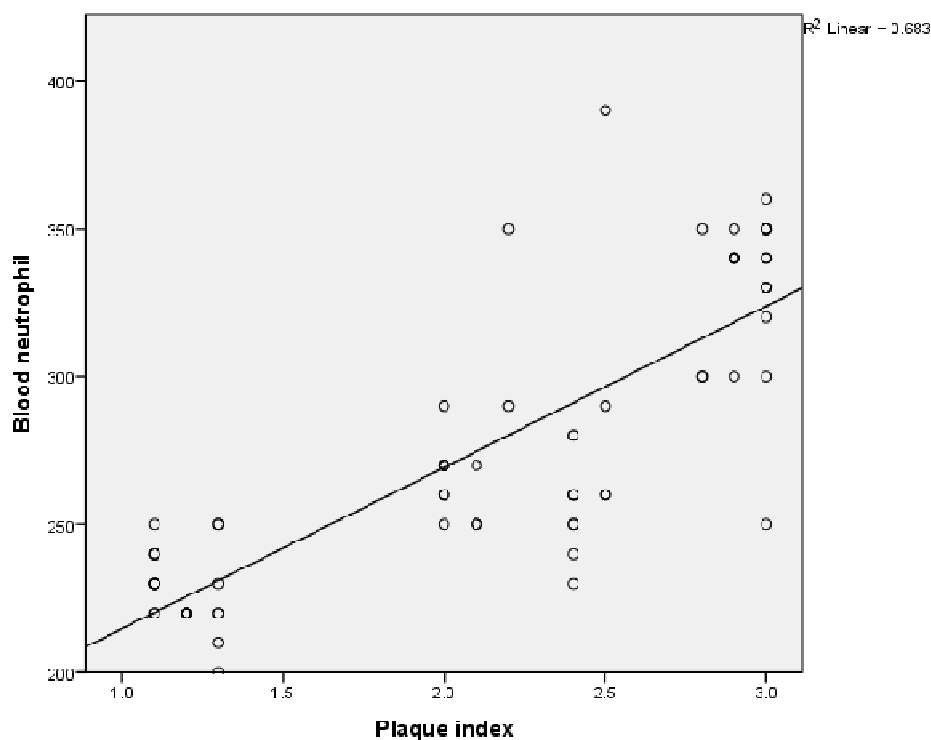
Figure-9-relationship between plaque index and blood neutrophil over three trimesters of pregnancy in experimental group.
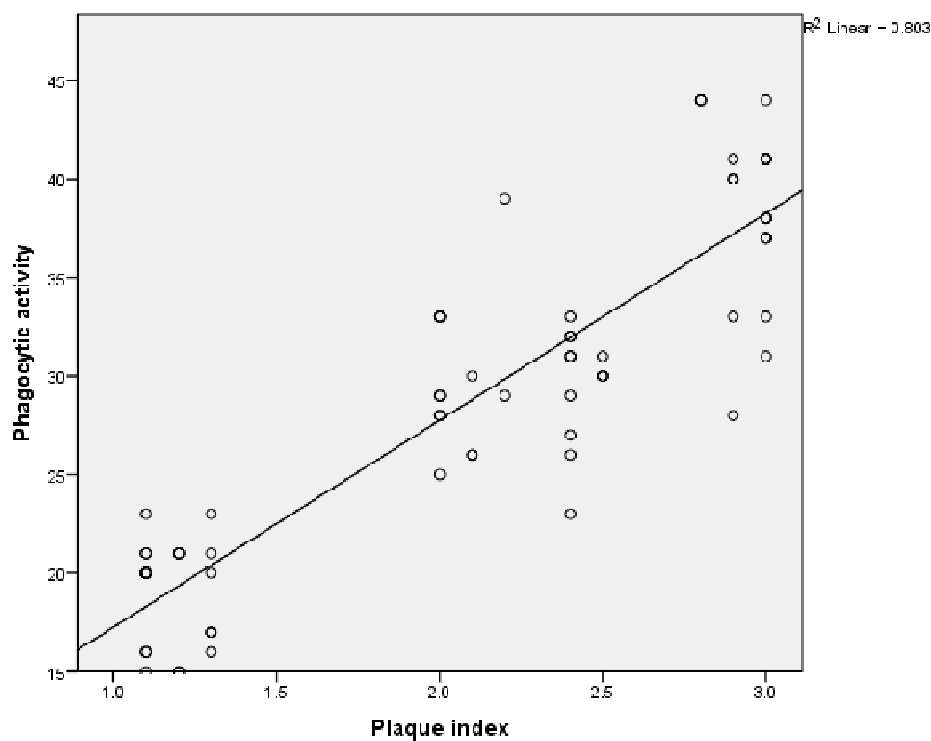

Figure-10-Relathioship between plaque index and phagocytic activity over three trimesters of pregnancy in experimental group.