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Expression of CD14+16+ and CD45RA Monocytes in Chronic and Aggressive Periodontitis: A Comparative Study

[1]Dr. Moumita Nayek, [2]Dr. Debabrata Kundu,
[3]Dr. Argha Rudra, [4]Dr. Debarshi Jana
[1](Assistant Professor, Bardwan Dental College And Hospital, Dept. Of Periodontics),
[2](Professor, North Bengal dental college)
[3](Associate professor, North Bengal dental college)
[4](Young Scientist, Department of Gynecology and Obstetrics Institute of Post-Graduate Medical Education and Research) Ph No- 9836002686
**Corresponding Author: Dr. Debarshi Jana*

ABSTRACT:- Peripheral blood monocytes are a heterogeneous cell population, with phenotypes that change on activation or differentiation. We have to find out the expression of monocyte subsets (CD14+CD16+ and CD45RA) in peripheral blood of patients suffering from aggressive and chronic periodontitis and to compare with that of the normal healthy individuals.
Total thirty subjects were selected for the study. The study model consisted of three groups – Group I, Group II and Group III. The subjects were allocated in the following groups as follows:
Group I: Normal healthy control, having clinically healthy periodontium. Ten subjects were included in this group.
Group II: Patients suffering from Chronic Periodontitis. All together ten subjects were taken in this group.
Group III: Patients having Aggressive Periodontitis. Ten patients were included in this group.
In group I, the mean CD14+CD16+ (mean± s.d.) of patients was .7327 ± .0794.In group II, the mean CD14+CD16+ (mean± s.d.) of patients was 4.3403 ± 1.7132.In group III, the mean
CD14+CD16+ (mean± s.d.) of patients was 2.7650 ± 1.4720.Difference of mean
CD14+CD16+ vs. three groups was statistically significant (p<0.0001). In group I, the mean
CD45RA (mean± s.d.) of patients was .3923 ± .0904.In group II, the mean CD45RA (mean±
s.d.) of patients was 1.8000 ± .6103.In group III, the mean CD45RA (mean± s.d.) of patients was 2.2587 ± 1.4110.Difference of mean CD45RA vs. three groups was statistically significant (p<0.0001).
The results show that the mean percentage of CD14+CD16+ monocytes was the maximum in the peripheral blood of chronic periodontitis patients followed by aggressive periodontitis and the healthy subjects respectively. The percentage of CD45RA level was increased in chronic periodontitis group as well as aggressive periodontitis group in comparison to the healthy group but the percentage was more in aggressive periodontitis patients.

Key Words: Chronic, Aggressive, Periodontitis monocyte subsets, CD14+CD16+, CD45RA

I. INTRODUCTION

Periodontitis is an inflammation of the supporting structure of teeth and is classified mainly as chronic and aggressive forms. The majority of the world's population is affected by periodontal disease (Russell & Kostlan 1961)[1]. The disease is destructive in nature and practically affects all dentate individuals and is the leading cause of tooth loss in many adult populations (Warehouse 1966) [2].

Monocytes/macrophages play a role in many pathological conditions including inflammation, infections, autoimmune disorders, atherosclerosis and display a great phenotypical and functional heterogeneity. In human blood, monocyte subpopulations are of two types: (Mizuno et al. 2005)[3]

a) The classical monocyte is characterized by high level expression of the CD14 cell surface receptor (CD14++ CD16- monocyte)

b) The non-classical monocyte shows low level expression of CD14 and with additional co-expression of the CD16 receptor (CD14+CD16+ monocyte).

After stimulation with microbial products the CD14+CD16+monocytes produce high amounts of pro-inflammatory cytokines like tumor necrosis factor and interleukin-12. In human blood, two monocyte populations can be distinguished, i.e., the CD14++CD16-DR+ classical monocytes and the CD14+CD16+DR++ Proinflammatory monocytes (Kai-Uwe Belge et al. 2002) [4]. In healthy subjects 90% of monocytes are

CD14++CD16- whereas a smaller population is CD14+CD16+. The latter represent more mature monocytes with proinflammatory capacities.[4]

A high count of CD14+CD16+ monocytes is found in severe infection (sepsis) (Fingerle et al. 1993)[5] and a very low count of these cells is found after therapy with immuno-suppressive glucocorticoids (Fingerle-Rowson et al. 1998) [6].

In patients with severe bacterial sepsis, amount of CD14+CD16+ monocytes seem to be markedly increased(500 cells/ul) from the average normal count (50 cells/ul) (Fingerle et al. 1993)[5]. Therefore it appears that the CD14+ CD16+ monocytes can be crucial players in infection and inflammation.

CD45 is a cell surface glycoprotein with a cytoplasmic tyrosine phosphatase domain. Spliced isoforms of CD45 are expressed on a restricted group of cells, and are designated CD45R. CD45RA is the high molecular weight isoform of CD45 (Clark 1989). In vitro activation of peripheral blood mononuclear cells induces CD45RA expression on monocytes (Brohee 1992)[7], and expression of CD45RA has been used as a marker of monocyte activation in vivo (Rothe et al. 1996)[8]. Examination of CD45RA on monocytes might help to detect the activation of circulating monocytes in periodontitis patients.

Understanding of the role and fate of functional subsets of circulating monocytes in vivo is an important and long-standing issue. Information about monocyte subsets and their functions may impact our understanding of diseases and the design of therapeutic strategies.

But, alteration of monocyte subsets in periodontitis patients has not been completely understood. Therefore, the present study has been undertaken to assess expression of CD14, CD16 and CD45RA on monocytes from patients with chronic and aggressive periodontitis as well as healthy subjects in order to determine the relationship between monocyte subsets and periodontitis.

Peripheral blood monocytes are a heterogeneous cell population, with phenotypes that change on activation or differentiation. Most of the monocytes express lipopolysaccharide (LPS) receptor, CD14 intensely, and do not express Fc γ receptor III, CD16 (CD14++CD16– monocytes). But the cell count of monocyte expressing CD16 with CD14 expression (CD14+CD16+ monocytes) increase in inflammatory diseases as well as in sepsis and bacteremia in hemodialysis patients.CD45RA is expressed on activated monocytes, and is regarded as an activation marker of peripheral blood monocytes. Periodontitis is an inflammatory condition but the alteration of monocyte subsets in periodontitis patients has not been completely understood to date. With this perspective the present study has been undertaken.

The objective of this study is

1. To find out the expression of monocyte subsets (CD14+CD16+) in peripheral blood of patients suffering from aggressive and chronic periodontitis and to compare with that of the normal healthy individuals.
2. To find out the expression of monocyte subsets (CD45RA) in peripheral blood of patients suffering from aggressive and chronic periodontitis and to compare with that of the normal healthy individuals.

II. METHOD

The present study was a short term, bi-centric study, carried out in the Department of Periodontics (Post Graduate Division), Dr. R .Ahmed Dental College and Hospital, Kolkata-700014, in association with Institute of Haematology &Transfusion Medicine, Kolkata- 73 under approved protocol.

Study Design:

Total thirty subjects were selected for the study. The study model consisted of three groups – Group I, Group II and Group III. The subjects were allocated in the following groups as follows:

Group I: Normal healthy control, having clinically healthy periodontium. Ten subjects were included in this group.

Group II: Patients suffering from Chronic Periodontitis. All together ten subjects were taken in this group.

Group III: Patients having Aggressive Periodontitis. Ten patients were included in this group.

Inflammation measured using GINGIVAL INDEX:

The severity of gingivitis was scored on four gingival scoring units (distal-facial papilla, facial margin, mesial-facial papilla and lingual gingival margin) of all teeth. A blunt instrument, a periodontal probe, was drawn horizontally along the soft tissue wall of entrance of the gingival sulcus for measuring purpose.

Scoring Criteria:

0=Normal gingiva

1=Mild inflammation slight change in color, slight edema, no bleeding on probing.

2=Moderate inflammation, redness, edema, glazing and bleeding on probing.

3=Severe inflammation, marked redness and edema, ulceration, spontaneous bleeding.

Gingival index score = (Total score)/(No.of surfaces examined)

GI score Interpretation
0.1 – 1.0 Mild Gingivitis
1.1 - 2.0 Moderate Gingivitis
2.1 - 3.0 Severe Gingivitis

CALCULUS INDEX- Simplified (CI-S)

This index system included only six teeth that were representatives of all anterior and posterior segments of mouth.

The facial surfaces of teeth #3, 8, 14, 24(universal system of notation) and lingual surfaces of teeth #19, 30(universal system of notation) were examined.

The scoring method for CI-S was as follows:

0= No calculus present.

1= Supragingival calculus covering not more than one third of the exposed tooth surface.

2= Supragingival calculus covering more than one third but not more than two-third of the exposed tooth surface or the presence of individual flecks of subgingival calculus around the cervical portion of the tooth or both.

3= Supragingival calculus covering more than two-third of the exposed tooth surface or a continuous heavy band of subgingival calculus around the cervical portion of the tooth or both.

A mouth mirror and a No.23 dental explorer were used to estimate the surface area covered by supragingival calculus and to explore for subgingival calculus. The dental explorer No.23 was gently placed into the distal contact area and walked to the mesial contact area. The facial surface included half of the mesial and distal surfaces.

The CI-S score for the individual was obtained by totaling the calculus score per tooth surface and dividing it by the number of surface examined.

PROBING POCKET DEPTH – PPD

It is the distance between the base of the pocket and the gingival margin. The distance was calculated with UNC-15 probe held parallel to the vertical axis of the tooth. The pocket depth was measured at 4 sites per tooth (mesiobuccal, buccal, distobuccal and lingual).

FACS Analysis:

The expression of CD14, CD16 and CD45RA on surface of monocytes were measured by using flow cytometry and analyzed by using Cell Quest Pro software. Twenty thousand events were counted for each sample (Fig29, Fig.30).

Statistical Analysis:

For statistical analysis data were entered into a Microsoft excel spreadsheet and then analysed by SPSS 24.0. and Graph Pad Prism version 5. A chi-squared test ($\chi 2$ test) was any statisticalhypothesis test wherein the sampling distribution of the test statistic is a chi-squared distribution when the null hypothesis is true. Without other qualification, 'chi-squared test' often is used as short for Pearson's chi-squared test. Unpaired proportions were compared by Chi-square test or Fischer's exact test, as appropriate. p-value ≤ 0.05 was considered for statistically significant.

III. RESULT

In group I, the mean gingival index (mean± s.d.) of patients was .0000 ±.0000.In group II, the mean gingival index (mean± s.d.) of patients was 2.4630 ± .2210.In group III, the mean gingival index (mean± s.d.) of patients was 1.1350 ± .3296.Difference of mean gingival index vs. three groups was statistically significant ($p<0.0001$).

In group I, the mean plaque index (mean± s.d.) of patients was .7880 ± .1047.In group II, the mean plaque index (mean± s.d.) of patients was 2.6790 ± .8951.In group III, the mean plaque index (mean± s.d.) of patients was 1.3430 ± .1092.Difference of mean plaque index vs. three groups was statistically significant ($p<0.0001$).

In group I, the mean probing pocket depth (mean± s.d.) of patients was 2.1667 ± .3527 mm.In group II, the mean probing pocket depth (mean± s.d.) of patients was 7.5273 ± .8388 mm.In group III, the mean probing

pocket depth (mean± s.d.) of patients was 5.2777 ± 1.1614 mm.Difference of mean probing pocket depthvs. three groups was statistically significant (p<0.0001).

In group I, the mean clinical attachment level (mean± s.d.) of patients was .0000 ± .0000 mm.In group II, the mean clinical attachment level (mean± s.d.) of patients was 4.6467 ± 1.0747 mm.In group III, the mean clinical attachment level (mean± s.d.) of patients was 3.6670 ± .7742 mm.Difference of mean clinical attachment levelvs. three groups was statistically significant (p<0.0001).

In group I, the mean CD14+CD16+ (mean± s.d.) of patients was .7327 ± .0794.In group II, the mean CD14+CD16+ (mean± s.d.) of patients was 4.3403 ± 1.7132.In group III, the mean CD14+CD16+ (mean± s.d.) of patients was 2.7650 ± 1.4720.Difference of mean CD14+CD16+ vs. three groups was statistically significant (p<0.0001).

In group I, the mean CD45RA (mean± s.d.) of patients was .3923 ± .0904.In group II, the mean CD45RA (mean± s.d.) of patients was 1.8000 ± .6103.In group III, the mean CD45RA (mean± s.d.) of patients was 2.2587 ± 1.4110.Difference of mean CD45RA vs. three groups was statistically significant (p<0.0001).

Table: Distribution of Mean GINGIVAL INDEX, PLAQUE INDEX, PROBING POCKET DEPTH and CLINICAL ATTACHMENT LEVELin Three Groups

		Number	Mean	SD	Minimum	Maximum	Median	p-value
GINGIVAL INDEX	Group I	30	.0000	.0000	0.0000	0.0000	0.0000	<0.0001
	Group II	30	2.4630	.2210	2.1700	2.7800	2.4300	
	Group III	30	1.1350	.3296	0.5000	1.5000	1.2750	
PLAQUE INDEX	Group I	30	.7880	.1047	0.5700	0.9500	0.8000	<0.0001
	Group II	30	2.6790	.8951	2.2100	7.3100	2.6000	
	Group III	30	1.3430	.1092	1.1100	1.5000	1.3050	
PROBING POCKET DEPTH(mm)	Group I	30	2.1667	.3527	1.5000	3.0000	2.2000	<0.0001
	Group II	30	7.5273	.8388	6.4500	8.8000	7.5000	
	Group III	30	5.2777	1.1614	3.6500	7.4000	4.9800	
CLINICAL ATTACHMENT LEVEL(mm)	Group I	30	.0000	.0000	0.0000	0.0000	0.0000	<0.0001
	Group II	30	4.6467	1.0747	2.5000	6.4000	4.5000	
	Group III	30	3.6670	.7742	2.3300	4.5500	3.5600	

Table: Distribution of Mean CD14+CD16+, CD45RAin Three Groups

		Number	Mean	SD	Minimum	Maximum	Median	p-value
CD14+CD16+	Group I	30	.7327	.0794	0.6100	0.8900	0.7300	<0.0001
	Group II	30	4.3403	1.7132	1.3200	7.5300	4.4400	
	Group III	30	2.7650	1.4720	1.1000	6.2100	2.2550	
CD45RA	Group I	30	.3923	.0904	0.3000	0.8100	0.3800	<0.0001
	Group II	30	1.8000	.6103	0.5600	3.2100	1.7950	
	Group III	30	2.2587	1.4110	0.9500	6.0400	1.9000	

IV. DISCUSSION

The accurate quantitative detection of different cell types and their subtypes infiltrating the periodontal lesion has been gaining momentum in recent years with the advancement of sophisticated instruments like flowcytometry and immunohistochemistry technology and the set of Cluster of Differentiation (CD) antigen specific monoclonal/ polyclonal antibodies.

In various systemic inflammatory conditions (i.e., rheumatoid arthritis, Atherosclerosis, Kawasaki disease and bacterial septicemia), percentage of CD14+16+ monocytes has been found to be increased which express high levels of mRNA for proinflammatory cytokines (IL-1,IL-6,TNF-α) and low levels of mRNA for anti-inflammatory cytokines. CD45RA is mainly expressed on CD14+CD16+ monocytes (Nagasawa et al. 2004)[9]. As periodontitis is an inflammatory condition caused by bacteria, percentage of these cells is likely to be altered in peripheral blood. Therefore the present study has been undertaken to find out the percentage of CD14+CD16+ monocytes and CD45RA monocytes involved in both chronic periodontitis and aggressive periodontitis and to compare them with that of healthy subjects using flowcytometry.

In the present study, mean percentage of CD14+CD16+ monocytes was found to be maximum in number in the peripheral blood of chronic periodontitis patients followed by aggressive periodontitis and the healthy subjects respectively (Table no 14). This finding was in accordance with the observation of Nagasawa et al. in 2004[9]. CD14+CD16+ monocytes are regarded to be more mature than CD14++CD16– monocytes as the majority of monocytes do not express CD16 (Grage-Griebenowet al.1993)[10], whereas tissue macrophages express CD16. This was supported by in vitro maturation studies, which revealed that CD14+CD16+ monocytes

could be generated from CD14++CD16– peripheral blood monocytes (Ziegler-Heitbrock et al. 1993)[11]. It has been reported that CD14+CD16+ monocytes were induced after repeated injection of LPS (Mackensen et al. 1992)[12]. Similarly, macrophage colony-stimulating factor (M-CSF) treatment induced CD14+CD16+ monocytes, and the effect was enhanced by combined M-CSF/interferon-c injection (Weiner et al. 1994)[13]. The increase of CD14+CD16+ monocytes in chronic periodontitis patients suggests that LPS, peptidoglycans, lipoteichoic acid of periodontopathogen might induce cytokines to develop CD14+CD16+ monocytes. Alternatively, cytokines released from a periodontal lesion might activate CD14++CD16– monocytes to differentiate into CD14+CD16+ monocytes. Nockher and Scherberich in 1998[14] found that percentage of CD14+CD16+ monocytes in renal failure patients undergoing haemodialysis, suffering from chronic infection constituted 24% of all blood monocytes. Katayama et al. in 2000[15] observed a high percentage of CD14+CD16+ monocytes in Kawasaki disease. In the present study, percentage of CD14+CD16+ monocytes in Chronic Periodontitis Group was 4.408±1.8525 and in Aggressive Periodontitis Group was 2.781±1.5819. This relatively lower percentage of CD14+CD16+ monocytes found in Chronic Periodontitis and in Aggressive Periodontitis Group may be due to the fact that periodontitis is a localized inflammatory condition which has a limited reflection in the systemic circulation.

The activation-associated expression of CD45RA, another marker of monocyte heterogeneity, was increased in patients with periodontitis (Table 18). As CD45RA is mainly expressed on CD14+CD16+ monocytes (Gabriel et al. 1994)[16], CD45RA+ monocytes might be an activated subset of CD14+CD16+ monocytes. In the present study, percentage of CD45RA level has been found to be increased in chronic periodontitis group and aggressive periodontitis group in comparison to the healthy group but the percentage was more in aggressive periodontitis patients (Table no 17). The finding was corroborated with the observation of Nagasawa et al. in 2004.[9]They proposed that increase of CD45RA+ monocytes in aggressive periodontitis patients might be related to genetic factors including apoE polymorphism. Moreover, it was reported that CD14+CD16+ and CD45RA+ monocytes were positively correlated to the total cholesterol and low-density-lipoprotein, respectively (Rotheet al.1996)[8]. They suggested that CD45RA+ expression is an indicator of monocyte activation in the presence of atherogenic lipoproteins. However, our study was not designed to find out the correlation between CD45RA expression and serum lipid profile. In our study, the statistically significant percentages of CD14+CD16+ monocytes and CD45RA+ monocytes were strongly correlated in aggressive periodontitis patients (r=0.93), but the strength of correlation was less in chronic periodontitis patients (r=0.54).It indicates that various stimuli (cytokines, genetic factors), pathogens and mechanisms might be involved in the control of differentiation of monocyte subsets in aggressive and chronic periodontitis patients in different ways, as also reported by Nagasawa et al. 2004[9].

In this study, different clinical parameters like Plaque Index (PI), Gingival Index (GI), Probing Pocket Depth (PPD) and Clinical Attachment Level (CAL) have been used to measure the periodontal condition of the subjects of different groups. In 1965 Löe et al.[17] used GI and PI for their pioneer work-'Experimental gingivitis in men' for the assessment of inflammatory condition of gingiva as well as the amount of plaque deposition. In 1978, Loesche and Syed also used PI and GI for assessment of gingivitis. Epidemiological data have shown that there is a close relationship between periodontal destruction and oral debris (Lövdal et al. 1958)[18]. Therefore, these two parameters have been used in the present study. In our study, the correlation between the CD14+CD16+ and clinical parameters such as Plaque Index (r=0.81), Gingival Index (r=0.69), Probing Pocket Depth (r=0.65) in chronic periodontitis group was found to be strongly correlated and was statistically significant but CAL (r=0.51) was less strongly associated with CD14+CD16+ levels (Table 21). Previously researchers have reported that CD14+CD16+ monocytes were mainly involved in the production of higher level of tumor necrosis factor-alpha (TNF-α) and IL-6 compared to CD14++CD16– monocytes (Belge et al. 2002)[19]. Therefore the strong correlation between PI,GI and PPD with CD14+CD16+ have been assumed to be associated with the inflammatory mediators that are indicators of current inflammatory disease activity. Whereas, CAL represents the individual's cumulative effect of present as well as past periodontal destruction and in our study some of the sites probably may not have been active at the time of the examination, thus CAL has been less strongly correlated with CD14+CD16+. However, the actual reason behind this particular observation is unclear.

The correlation between the CD45RA and clinical parameters such as Plaque Index and Gingival Index was found to be statistically significant in chronic periodontitis group but they were less strongly correlated (r =0.51for PI ,r =0.56for GI). The strength of association between PPD and CD45RA was medium(r=-0.42) but negatively correlated.CAL was found to be weakly correlated with CD45RA(r=0.31) (Table 21). Although, CD45RA has been shown to be expressed on activated CD14+CD16+ monocytes (Gabriel et al. 1994)[16] but the negative and weak correlation of PPD and CAL respectively with CD45RA expression may be due to incorporation of clinical parameters which reflects the past disease experience. However, this finding in our

study raises a question that whether it is rational to search for a correlation between locally measured clinical parameters and CD45RA expression on the blood monocytes of systemic circulation.

In aggressive periodontitis patients, CD14+CD16+ and CD45RA were found to be weakly correlated with clinical parameters like PI and GI (Table 21). The results indicate the lack of clinical inflammation and plaque accumulation in localized aggressive periodontitis patients, despite the presence of deep periodontal pocket and advanced bone loss. This might look to be inconsistent with the amount of periodontal destruction but is an established fact (Lang et al. 1999)[20]. The patients selected for the aggressive periodontitis group in our study were mostly suffering from localized aggressive periodontitis leading to the mean value of PI and GI weakly correlating with both the markers. Moreover, the lack of inflammatory conditions can also be found in generalized aggressive periodontitis patients that coincide with a period of quiescence in which low score of GI are usually seen (Page & Schroeder 1982)[21].

In aggressive periodontitis patients, CD14+CD16+ and CD45RA were strongly but negatively correlated with PPD (Table no.21). The actual reason for such reverse correlation remains unclear. However PPD does not always reflect the severity of current destruction in periodontitis patients.

V. CONCLUSION

Peripheral blood of 10 chronic periodontitis patients, 10 aggressive periodontitis patients and 10 healthy subjects were investigated for the expression of CD14+CD16+ monocytes and CD45RA monocytes by flow cytometry.

The results show that the mean percentage of CD14+CD16+ monocytes was the maximum in the peripheral blood of chronic periodontitis patients followed by aggressive periodontitis and the healthy subjects respectively. The percentage of CD45RA level was increased in chronic periodontitis group as well as aggressive periodontitis group in comparison to the healthy group but the percentage was more in aggressive periodontitis patients.

Since this is a pioneer study in this geographic region, further studies with larger sample sizes and with more sophisticated technology are warranted to arrive at a definitive conclusion.

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Corresponding Author: Dr. Debarshi Jana
Young Scientist (DST) Department of Gynecology and Obstetrics Institute of Post-Graduate Medical Education and Research, A.J.C. Bose Road, Kolkata-700020, West Bengal, India Ph No- 9836002686