Changes in Biochemical Parameters Associated with Periodontal Disease

IONUT LUCHIAN[1], IOANA MARTU[1]*, CRISTIAN MARTU[2]*, ANCUTA GORIUC[1], ANTONELA BELDIMAN[1], SILVIA MARTU[1]

[1] Grigore T. Popa University of Medicine and Pharmacy Iasi, Faculty of Dental Medicine, 16 Universitatii Str., 700115, Iasi, Romania

[2] Grigore T. Popa University of Medicine and Pharmacy Iasi, Faculty of Medicine, 16 Universitatii Str., 700115, Iasi, Romania

Periodontitis is an inflammatory disease caused by specific bacteria present in the oral cavity. Several mechanisms have been proposed in order to explain the disease's pathogenesis. Among them, the most common and intensely studied is the neutrophil dysfunction with a reduction in the chemotaxic function, that is significantly influenced by the intercellular signalling pathways via inflammatory cytokines. In this study, we assayed serum IL-8, TNF-α and IFN-γ,from patients with aggressive and chronic periodontitis and compared them with healthy controls. We found a significantincrease in TNF-α levels that was higher in the aggressive versus chronic periodontitis. IFN-γ was also increased in chronic periodontitis. There was no diffference in IL-8 levels between healthy and diseased patients. These results indicate that TNF-α can be used as a potential marker in periodontal disease

Keywords: Cytokine, periodontal diseases, interleukins, IL-8, TNF-α, IFN-γ

Periodontitis is an inflammatory disease caused by specific bacteria located in the oral cavity. It has been classified in aggressive periodontitis (Ag-P) and chronic periodontitis (Cr-P), with age of onset for Ag-P being much lower than that for Cr-P. Ag-P has been associated with neutrophil dysfunction [1-5], singular nucleotide polimorfism [1, 6], and specific imun response to infectious agents [1, 7]. Previuos studies have investigated the neutrophil disfunction, in order to decipher chemotaxic mechanism. Several mechanisms have been proposed to explain this pathology that include: diacilglicerol accumulation, reduction in action for DAG kinase, reduction in activity for protein-kinase, rise in nitric oxide synthesis, rise in super-oxide production and reduction in calcium influx. There also many studies conducted on the influence of different parameters in Periodontal Disease [8-10].

Immune cells within the periodontium release proinflamatory cytokines in response to periodontal pathogens and their toxins. These cytokines are produced by activated T lymphocytes and can be found in high concentrations in gingival fluid and tissues affected by the disease.

Th1 lymphocytes produce IFN-γ and TNF-α, while Th2 produce Il-4, 5, 6 and 13 and these are essential in antibody production and eradication of the extracelullar microorganisms.

Previous studies have reported a rise in IFN-γ serum leveland gingival fluid in the periodontic patient compared to the healthy one [11-14]. Therefore, to determine whether specific inflammatory markers are associated with periodontal disease we compared serum IL-8, TNF-α, and IFN-γ levels of patients with agressive and chronic form of periodontitis with those of healthy individuals

Experimental part

A sample population of 42 patients was included in our study having the ages between 24 and 55. Venous blood was colected and IL-8, TNF-α, IFN-γ levels were determined using ELISA imunoassay tehnique. For IL-8 assay an ELISA kit was used produced by R&D Systems Inc. USA. Etalon curve was constructed and all incubations were done at room temperature. Enzyme used was HRP that has H_2O_2 as a substrate. Reaction between enzyme and substrate generates a blue colour. The reaction is than stopped with sulfuric acid 2N when it generates a yellow product measured at 450 nm. IFN-γ was measured in the same manner.

Results and discussions

In the 42 group of pacients 22 were males and 20 females. This group was distributed in 3 subgroups:

Subgroup A - 16 patients with chronic periodontitis.

Subgroup B - 11 patients with aggressive periodontitis.

Subgroup C - control group, consists of 15 patients with no periodontal disease.

IL-8 level statistical analysis for the control group showed a mean level of 0.54 ± 0.21pg/mL, while maximum level for this cytokine within the healthy patient group was 0.87pg/mL. In the aggressive parodontits group, the IL-8 mean level was 0.62 ± 0.23pg/mL, with a maximum value of 0.96pg/mL.

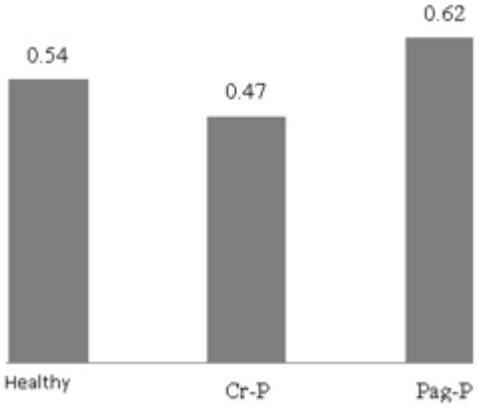

Fig.1. Il-8 levels in healthy, Chronic and aggressive periodontitis (pg/mL)

The distribution of Il-8 levels in the 3 subgroups was as in table 1.

The statistical analysis for the 3 subgroups has not showed a significant difference between the healthy and Cr-P (table 2).

TNF-α level was presented in table 3.

* email: ioanamartu@gmail.com; cristimartu@gmail.com

Patient group	Patients	IL-8 mean value (pg/mL)	Standard deviation (pg/mL)	IL-8 minimum serum value (pg/mL)	IL-8 maximum serum value (pg/mL)
Healthy	15	0.54	0.21	0.01	0.87
Cr-P	16	0.47	0.18	0	0.89
Ag-P	11	0.62	0.23	0	0.96

Table 1
IL-8 LEVELS PATIENT GROUP DISTRIBUTION

Age group	Number	Il-8 mean value	Standard deviation
24-30	6	0.35	0.62
30-40	12	0.42	0.27
>40	24	0.56	1.23

Table 2
IL-8 AGE RELATED LEVELS

Group	Number	TNF-α mean value (pg/mL)	STD	TNF- α serum minimum value (pg/mL)	TNF- α maximum serum value (pg/mL)
Healthy	15	1.3	0.54	0	2.4
Cr-P	16	2.4	1.21	0	4.8
Ag-P	11	3.2	0.72	0	5.7

Table 3
TNF-α LEVEL SUBGROUP DISTRIBUTION

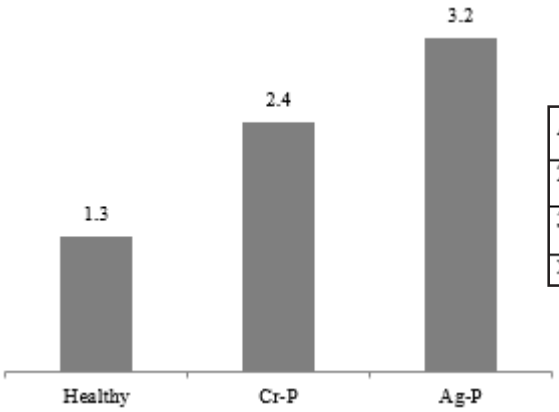

Fig. 2. TNF-α level for healty, chronic and aggressive periodontitis (pg/mL)

Table 4
EFFECTS OF AGE ON TNF-α LEVELS

Age group	Number	TNF-α mean value	Standard deviation
24-30	6	0.67	0.45
30-40	12	1.97	0.86
>40	24	2.3	1.45

Group	Number	IFN-γ mean value (pg/mL)	STD	IFN- γ serum min value (pg/mL)	IFN- γ max serum value (pg/mL)
Healthy	15	67.32	12.34	0.3	89.34
Cr-P	16	99.56	24.56	0.87	121.3
Ag-P	11	85.69	8.9	0.65	105.8

Table 5
IFN-γ LEVELS IN HEALTHY CR-P AND AG-P

Age group	Number	IFN- γ mean value	Standard deviation
24-30	6	69.75	2.5
30-40	12	78.65	13.45
>40	24	87.63	11.54

Table 6
IFN-γ AGE RELATED LEVELS

There was a significant increase in TNF-α levels in chronic and aggressive periodontitis with TNF-α levels being the highest within the Ag-P group.

There was a significant effect of age on TNF-α levels with the most significant increase in TNF-α levels occurring in the group over 40-year-old.

For IFN-γ levels the mean value in the healthy group was 67.32 pg/mL with a standard deviation of 12.34. For patients with Ag-P mean value was 85.69 pg/mL± 8.9 and for those with Cr-P the mean value was 99.56 pg/mL ± 24.56 (table 5).

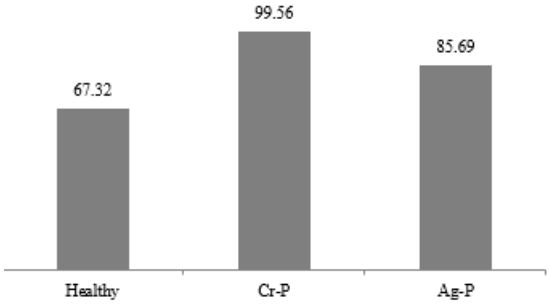

Fig. 3. IFN-γ level healhty, Chronic and aggressive periodontitis (pg/mL)

The statistical analysis for the IFN-γ levels in the 3 subgropus showed a significant difference between subgroups. The highest values for IFN-γ were in the Cr-P subgroup with a value of 121.3 pg/mL (table 6).

Conclusions

In this study we showed that periodontitisis associated with a significant increase in circulating markers of inflammation. As such circulating TNF-α was significantly higher inaggressive and chronic periodontitis. Similarly there was a significant increase in IFN-γ levels in patients with chronic periodontitis compared to healthy controls. These effects were significantly more pronounced in patients over 40 years old compared to younger ages. Finally, there was no change in IL-8 levels in periodontitis patients compared to healthy individuals. Together, these results show that TNF-α can be considered a strong candidate marker in periodontal disease

References

1.ADRIAENS, P.A., ADRIAENS, L.M., Periodontology 2000, **36**, 2004, p. 121.

2.DANIEL, M.A., McDONALD, G., OFFENBACHER, S., VAN DYKE, T.E., Journal of Periodontology, **64**, 1993, p .617.

3.KANER, D., CHRISTIAN, C., DIETRICH, T., BERNIMOULIN, J.P., KLEBER, B.M., FRIEDMAN, A., Journal of Periodontology, **78**, 2007, p. 1201.

4.KURATKO, C.N., BECKER, S.A., Pathogenesis, **1**, nr. 4, 2000, p. 245.

5.MARTU, S., NANESCU, S.E., CONSTANTIN, L., Romanian Journal of Dentistry, **10**, no. 1, 2006, p. 35.

6.SHAHRESTANI, P., MUELLER, L.D., ROSE, M.R., Current Aging Science, **2**, 2009, p. 3.

7.UITTO, V.J., OVERALL, C., McCULLOCH, C., Periodontology 2000, **31**, 2003, p. 77.

8.BOARIU, M., MARINESCU, A., KRALEV, A., CLONDA, C., NEGRUTIU, M.L., SINESCU, C., Rev. Chim. (Bucharest), **65**, no. 12, 2014, p. 1477.

9.VELEA, O.A., SINESCU, C., ZEICU, C., FREIMAN, P.C., VELEA, P.I., ONISEI, D., DUMA, V.F., Rev. Chim. (Bucharest), **65**, no. 9, 2014, p. 1063.

10.MANOLE, M., SINESCU, C., NEGRUTIU, M.L., ARDELEAN, L., DUDEA, D., ROMINU, M., Mat. Plast., **49**, no. 3, 2012, p. 163.

11.WONG, A., DUCIK-STEFANOVIC, S., GASIC-MILENKOVIC, J., European Journal of Neuroscience, **14**, no. 12, 2001, p. 1961.

12.WOODSIDE, J. V., McCALL, D., McGARTLAND, C., YOUNG, I.S., Proceed. of the Nutrition Society, **64**, 2005, p. 543.

13.XAJIGEORGIOU, C., SAKELLARI, D., SLINI, T., BAKA, A., KONSTANTINIDIS, A., Journal of Clinical Periodontology, **33**, 2006, p. 254.

14.XIMENEZ-FYVIE, L.A., HAFFAJEE, A.D., SOKRANSKY, S.S., Journal of Clinical Periodontology, **27**, no. 9, 2000, p. 648.

Manuscript received: 2.12.2015