Neutrophil Function in 8 Cases of Papillon-Lefevre Syndrome

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Statement of Problem: Papillon Lefevre syndrome (PLS) is a rate autosomal recessive disorder, which is characterized by palmar- plantar hyperkeratosis and rapid periodontal destruction of primary and permanent dentitions.
Purpose: The aim of the present study was to evaluate the peripheral blood neutrophil function including random locomotion, chemotaxis and oxidative mechanism of killing in a group of patients with PLS.
Materials and Methods: Peripheral blood was obtained from 8 PLS patients and 92 healthy control subjects. PMN mobility was measured by a modification of the micromethod of Addison and Babbage. Latex-Stimulated NBT reduction test described by Park et al was followed. Data were analyzed by Mann Whitney U test.
Results: The chemotactic activity in the PLS group was significantly lower than control group (89.5±21.6 vs 113±16 μm, $P<0.002$). The rate of NBT reduction by PLS patients leukocytes was 50.6±14.9% in comparison with the control group (52.2±16.1%). The patients group showed a random locomotion rate of 46.5±10.4 μm. This value for the control group was 43.9±13.6 μm. Both oxidative mechanism of killing and random location were not significantly different from those of the healthy control subjects ($P>0.05$).
Conclusion: The present study indicated an impaired neutrophil chemotaxis in PLS patients.

Key words: Papillon Lefevre Syndrome - Chemotaxis - Neutrophils

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In 1924 Papillon and Lefevre described two siblings from a first-cousin mating, with condition characterized by diffuse transgradient palmoplantar keratosis and the premature loss of the deciduous and permanent teeth. The condition became known as Papillon-Lefevre syndrome (PLS) and subsequently more than 250 cases have been reported up to 1998.[1,2]

In the current classification of palmoplantar keratodermas, PLS is the type IV of palmo-plantar ectodermal dysplasia.[3] This syndrome is an autosomal recessive disease with unknown etiology. Genetic analysis in affected families has shown a linkae in the region of 11q14-q21.[2,4]

Recently, mutation of the cathepsin C gene has been identified as the underlying genetic defect in PLS.[5,6,7]

Although the mechanism of periodontal destruction is not known, a possibly impaired

immune response is suspected. Some reports of functional anomalies of polymorphonuclear (PMN) cells including chemotaxis, phagocytosis and intracellular killing support this idea. Defects in neutrophil chemotaxis have been reported by several investigators [8-14] whereas normal chemotaxis has been reported by others.[15-17]

Both defective random locomotion [9,13] and normal random locomotion [16,17] of neutrophils have been reported.

Reports on the phagocytic and bactericidal ability of PMNs in PLS patients also showed conflicting results.

Impaired neutrophil phagocytosis[8,12,18], normal or slightly increased phagocytosis[19], increased superoxide production [20], impaired superoxide production[12], reduced myeloperoxidase activity[11],defective[8,17]and normal intracellular killing activity of neutrophils against staphylococcus aureus [16,10] have been reported.

This study was designed to assess peripheral blood neutrophil functions including random locomotion, chemotaxis and oxidative mechanism of killing in a group of Iranian PLS patients.

Materials and Methods

Patients Group:

Patients group was consisted of 8 PLS patients (2 boys and 6 girls). They belonged to 7 families and their mean age was 11.3 years (range: 5-29).

They had not received any periodontal treatment or medication including antibiotics, anti-inflammatory and hormonal drugs during 6 months before entering to the study. Ninety-two healthy individuals with mean age of 15.5 years (range: 5-32) were also studied as the group control.

These individuals were checked for the presence of any oral or systemic disorders. Alcoholic or smoking people were not considered. Informed consent was obtained from all subjects/parents.

Sera Collection:

Seven ml of heparinized peripheral blood was collected from the patients and healthy controls at 8:00–10:00 am by venipuncture of the antecubital vein after 10 hours of fasting.

Neutrophil Chemotaxis Assay:

PMN mobility was measured by a modification of the micromethod of Addison and Babbage.[21] Briefly, leukocytes were separated by dextran sedimentation from 5 ml of heparinized peripheral blood from PLS patients and healthy controls. Cells were washed and the concentration was adjusted to 2×10^6 cells/ml in phosphate-buffered saline (PBS). Chemotactic factor was generated by adding 0.02 ml Escherischia coli endotoxin (1.5 mg/ml, Sigma, USA) and 0.1 ml of normal serum to 1 ml PBS. A Whatman disc (13 mm diameter) was placed into each wells of a plastic plate (Flow multidisc FB20). 0.2 ml of chemotactic reagent and 0.2 ml of PBS (as negative control for random locomotion) were applied to the wells. A membrane from pretested micropore filters (3 µm pore size, 13 mm diameter, Millipore, USA) was placed on each disc and onto this, a plastic cap filled with 0.25 ml of cell suspension was inverted. After appropriate incubation at 37°C, membranes were removed, fixed and stained with hematoxylin and methylenblue. The X40 objective of a microscope was adjusted on the membrane surface and focused down to the lowest level at which at least five cells were seen. The distance was measured on the micrometer of the microscope's fine adjustment. The mean of ten observations on each of duplicate membranes was taken.

Nitroblue tetrazolium dye reduction test:

Latex-stimulated NBT reduction test described by Park et al was performed.[22] Cells (10^6/ml) were incubated for 15 min at 37°C with equal volume of 0.2% NBT (Sigma, USA) in PBS. Then 50 µL latex particles (0.8 µm, Difco Laboratories, USA) were added.

After 15 minutes incubation at 37°C, the suspension was gently mixed and cell smears were prepared. The percentage of cells that engulfed latex particles and contained intracellular blue-black formazan deposits was determined on Wright-Giemsa-stained slide preparations. Two individuals assessed at least 200 cells.

Statistical analysis:

The results of the PLS group were compared statistically to those of the control group using Mann Whitney U test. The results were expressed as a significant level of $P<0.05$.

Results

Random locomotion, chemotaxis and NBT values of the PLS patients and control group are presented in Table I. The random locomotion value of the PLS group showed no significant difference with the control group [46.5± 10.4 *vs* 43.9±13.6 μm].

A statistically significant decrease ($P<0.002$) of peripheral blood neutrophils chemotaxis in PLS group (89.5±21.6 μm) was observed in comparison with control group (113±16 μm).

No significant difference in NBT reduction test was observed between patient and control groups (50.6±14.9% *vs* 52.2±16.1%).

Discussion

Studies of periodontal diseases have shown the important role of PMNs functions in defending against periodontal pathogens and preventing the periodountium from infection. In the present study the functional capacity of neutrophils including random locomotion, chemotaxis and NBT reduction in a group of patients with PLS was evaluated. It was found a significantly decreased chemotaxis of neutrophils in these patients compared to the control group. Various cases of PLS with depressed neutrophil chemotaxis have been reported. Firatli et al studied the chemotaxis of neutrophils in 7 patients with PLS using the zymosan activated serum assay and found that not only chemotaxis but also spontaneous migration was depressed in all the patients.[13] In another study PMNs from 2 cases of PLS showed depressed random locomotion and chemotaxis.[9]

In the present study a normal random locomotion of PMNs in all the patients was observed. In agreement to this result, random locomotion showed no decrease in two other studies.[16,17]

D'Angelo et al[11] have reported leukocyte chemotaxis deficiency in a three-year-old girl. In this patient, a reduction of myeloperoxidase content of neutrophils was also detected. In another study PMNs from 2 cases of PLS showed depressed chemotactic response to FMLP and IL-8.[14]

The mechanism of impaired chemotaxis in PLS patients is not known. Abnormal neutrophil chemotaxis can be occurred due to defects in cell adhesion molecules or cell activation and movement elements.[23,24] Cytokines such as TNF-α and IL-1β can enhance the expression of adherence molecules and down regulate FMLP receptors and decrease chemotaxis.[25,26] Serum elevation of these cytokines coincident with altered neutrophil function in juvenile periodontitis has been shown.[27,28] As, it has been reported, a complete absence of dipeptidyl peptidase I (cathepsin C) activity is the cause of PLS.[5] Cathepsin C has an essential role in the activation of granule serine proteases expressed in both lymphoid and myeloid cells.

Table I: Neutrophil function test in PLS patients and control group

Examination	PLS group (n=8)		Control group (n=92)		P. value
	Mean ± SD	Range	Mean ± SD	Range	
Random locomotion	46.5±10.4 μm	35-62	43.9±13.6 μm	20-86	NS
Chemotaxis	89.5±21.6 μm	57-122	113±16 μm	72-143	<0.002
NBT	50.6±14.9 %	37-80	52.2±16.1 %	32-89	N.S.

NS: Not Significant

These proteases are implicated in a wide variety of immune and inflammatory processes, including phagocytic destruction of bacteria and local activation or deactivation of cytokines and other inflammatory mediators. In a recent study, a mouse deficient in dipeptidyl peptidase I was generated.[29] Although these mice showed normal in vitro chemotaxis, they were resistant to induction of acute arthritis and also showed a defect in neutrophil recruitment. The decrease in inflammatory cell accumulation was accompanied by a decrease in local production of TNF-a and IL-1ß production supporting the hypothesis that neutrophil-derived serine proteases are involved in the regulation of cytokine production at sites of inflammation.

There are some reports indicating the correction of neutrophil chemotaxis function following treatment or spontaneously.

Bullon et al[12] reported impaired neutrophil chemotaxis in two sisters affected by PLS.[12] Neutrophil function was corrected in both sisters after scaling and root planning. Improvement of neutrophil chemotaxis defect in two patients was also reported spontaneously after 7 years.[30,23] In another study the improvement of the patient's PMN function was coincident with the lack of detection of certain periodontopathic bacteria.[31] It indicates that decreased chemotaxis might not be a primary cause but is secondary to other factors involved in the pathogenesis of the disease, e.g. soluble bacterial factors that their ability to modulate or block chemokine receptors have been shown.[32,33]

Despite of decreased chemotaxis observed in our patients, no defect in normal random locomotion as well as superoxide generating ability in neutrophils was found. The oxidative and non-oxidative process in neutrophils is important in host defense against anaerobic bacteria in the gingival crevice or tissue-invading bacteria. In most of the previous studies, a decreased neutrophil function has been reported. In a study on 15 cases of 4 Egyptian families,[18] the neutrophil phagocytic and lytic activity was impaired. In another study, the production of superoxide radicals by PMNs in addition to chemotaxis was significantly depressed in two sisters.[12]

In contrast to these reports and in agreement with ours, phagocytosis by polymorphonuclear cells and NBT reduction showed no decrease in two other studies.[16,19] It is indicating that the defect in proteins of the NADPH oxidase system and generation of superoxide is not etiologically involved in this group of patients.

Taken together, results of this study in conjunction with other reports showed the impaired phagocytic function particularly defect in the chemotaxis major role of neutrophils in host defense mechanism.

The possible role of such a defect in the pathogenesis of the disease and the mechanisms involved in diminished neutrophil function needs more investigations especially at the molecular level.

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