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Recurrent Pyogenic Skin Infections (A Study of Neutrophil functions)

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SUMMARY

This study included 65 patients and 35 healthy control persons of matched age and sex. The patients were sufferers of recurrent and extensive skin infections. The patients were subjected to the following laboratory tests: pus swab for culture, quantitative determination of immunoglobulins IgG, M, A and E, and complement C4, in vitro chemotaxis assay of neutrophils, and Nitro Blue Tetrazolium dye test. The results of the laboratory investigations are presented and their significance is discussed.

The host's primary defense mechanisms against microbial infection depend on phagocytosis and intracellular killing by neutrophils. The neutrophil is equipped with and assay of sensory, locomotor, and chemical mechanisms to perform its functions properly[1].

At sites of tissue invasion by micro-organisms, humoral factors are released which induce neutrophils to leave the blood stream and enter the tissues. Optimum levels of antibodies and complement are vital for proper antimicrobial action of neutrophils[2].

Neutrophil function disorders that predispose to recurrent bacterial infections may or may not be accompanied by other abnormalities of humoral and/or cellular immunity. Recurrent pyogenic infections of the skin are considered to be the commonest clinical manifestation of defective neutrophil function[3]. Recurrent pyogenic skin infections due to defective neutrophil functions do occur in patients with defective host defense[2]. Also, alteration of neutrophil functions seem to occur during bacterial infections in patients with normal host defense[4].

Materials and Methods

This study included 65 patients and 35 healthy control persons of matched age and sex. The patients were sufferers of recurrent and extensive skin infections. Patients with following criteria were included in the study:

1. Patients with pyogenic skin infection at the time of examination.
2. Patients with history of recurrent pyogenic infections of the skin with or without other sites as tonsillitis, Otitis media, Pneumonia, etc..., with a frequency of at least four attacks per year and inadequate response to appropriate antibiotic therapy.
3. All the patients should not be on antibiotic

treatment for at least two weeks prior to the time of examination.

Exclusion criteria included all patients with disorders known to affect the host resistance to infection such as diabetes, neutropenia, etc.

All patients were subjected to a detailed history that included age, sex, occupation, drug and alcohol intake, diabetes, personal and family history of recurrent pyogenic infections.

Physical Examination

Besides general medical examination, all the patients and controls were examined to exclude any infected skin lesion in the controls and to identify the clinical diagnosis, distribution and extension of lesions in the patients.

Laboratory tests

The following laboratory tests were carried out:

1. Routine laboratory tests, i.e. urine examination, stool examination and complete blood count.
2. Pus swab for culture (Patients only) were inoculated on Cystine Lactose-Electrolyte Deficient medium (CLED agar) for 24-48 hours at 37°C. The resultant calories were identified by its appearance, colour, surface, size, and gram stain examination.
3. Quantitative determination of immunoglobulins, IgG, M, A, E and complement C4 by the single radial immunodiffusion technique[5].
4. Chemotaxis assay of neutrophils by in vitro testing using Boyden chamber technique[6] for calculation of the leading front method (Zigmond and Hirsch) was used[7].
5. Nitro Blue Tetrazolium dye reduction test (NBT) using the method adopted by Gifford and Malawista[8] as modified by Sallam and Azmy.[9]

The results were evaluated using the Chi-squared test and student-T test for statistical comparison of the groups included in this study.

Table 1. The clinical diagnosis cutaneous pyogenic infections.

Diagnosis	Recurrent infections		Severe recurrent infections	
	No.	%	No.	%
Furunculosis	18	36	8	53.3
Impetigo	14	28	4	26.6
Abscess	9	18	-	-
Ecthyma	9	18	2	13.3
Folliculities	7	14	-	-
Erysipelas	-	-	1	6.6

Results

The groups of patients was divided into two groups; the first group (50 patients) with recurrent pyogenic skin infections and the second group (15 patients) with severe recurrent pyogenic skin infection. The latter group was characterized by more frequent attacks (more than six attacks per year) and by a very poor response to the antibiotic treatment.

Table 1 shows the clinical diagnosis of the cutaneous pyogenic infections among the two groups of patients. Table 2 shows the different micro-organisms isolated from patients in

Table 2. Micro-organisms isolated from cutaneous lesions among patients with recurrent infections.

Organisms	Recurrent infections		Severe recurrent infections	
	No.	%	No.	%
Staph. aureus	34	65.4	13.	86.6
Steph. pyogenes	3	5.7	1	6.6
Staph. epider.	10	19.2	1	6.6
Diphtheroids	4	7.4	-	-
-ve	1	1.9	-	-
Total	52	99.6	15	99.8

Table 3. The results of different laboratory investigations of both groups of patients and controls.

Test	Control (N=35)	Recurrent infections (N=50)	Severe recurrent infections (N=15)
Total leucocytic count	3600-10500/mm3 (6900+1553)	4600-18000 (8584+2603)	4800-13000 (8693+2498)
Absolute neutrophilic count	1961-7245.mm3 (4205+1068)	2301-10595 (5344+1990)	2784-10400 (5629+2073)
Absolute eosinophilic count	0-525/mm3 (255+147)	78-1368 (454-291)	78-1596 (546+398)
NBT	50-96% (73.8+13.7)	30-91 (66.1+13.8)	10-75 (52.9+17.9)
Chemotaxis	30-58mm (43.4+7.6)	27-52 (39.6+5.8)	20-40mm (28.2+6.2)
IgG	113-260 i.u/ml. (195.3+40.8)	62-384 (200+48.6)	14-244 (146.5+70.5)
IgM	117-391i.u/ml. (245+65.1)	63-443 (177.7+92.2)	24-286 (126.4+81.1)
IgA	63-262i.u/ml (118.5+57.4)	58-270 (146.4+50.5)	30-253 (105.5+66.4)
IgE	0-380i.u/ml (102.9+131.7)	0-3100 (67.46+1027.7)	0-4220 (870.7+1386)
C4	91.4-248 i.u/ml (144.9+43.3)	78.2+284 (157.8+40.5)	19.2-348 (212.8+123.5)

both groups. Table 3 shows the results of laboratory investigations of both groups of patients (i.e. with recurrent pyogenic skin infections and with severe recurrent pyogenic skin infections) and the control group, and Table 4 shows a comparison between the results of the different groups using the non parametric Mann-Whitney test.

Table 4. Comparison of the results of different groups using the non-parametric Mann-Whitney test.

Laboratory Test	Group l(n=50) compared to controls		Group 2(n=15) compared to controls		Group l (n=50) compared to Group 2 (n=15)	
	Z Value	**P Value**	**Z Value**	**P Value**	**Z Value**	**P Value**
1. Total leucocytic count/cmm	3.21	0.001	2.16	<0.05	0.24	>0.05
2. Absolute neutrophilic count/cmm	2.91	0.004	2.28	<0.05	0.30	>0.05
3. Absolute eosinophilic count/cmm	3.60	<0.001	3.55	0.001	0.71	>0.001
4. NBT %	2.24	<0.05	3.55	<0.001	2.46	<0.02
5. Chemotaxis (nm)	2.13	<0.05	4.63	<0.001	4.69	<0.001
6. IgM iu/ml	4.75	<0.001	4.10	<0.001	1.77	>0.05
7. IgG iu/ml	0.14	>0.05	2.34	<0.05	2.45	<0.05
8.IgA iu/ml	2.85	<0.05	0.97	>0.05	2.93	>0.05
9.IgE iu/ml	2.79	<0.005	2.16	<0.05	0.06	>0.05
10. C4 iu/ml	2.07	<0.05	1.84	>0.05	1.94	<0.05

P<0.05 indicates statistically significant difference.

Discussion

The Boyden chamber technique assay for chemotaxis is a good indicator for the sensory and locomotor behavior of the neutrophils and also for their adherence[6]. The nitroblue tetrazolium dye reduction slide test determines the phagocytic and bactericidal activities of the neutrophil and is indicative for its adherence pwoer[10]. The assessment of serum immunoglobulins and C4 reflects the chemotactic and opsonic activity of the serum[11].

The results of the laboratory investigations of patients with recurrent pyogenic skin infections showed increase in the total leucocytic count and the absolute Neutrophilic count indicating that the quantitative response is intact. This neutrophilia is due to the mobilization of the marrow neutrophil reserve in response to infection[1]. The presence of eosinophilia in a good percentage of the patients (28%) may be explained by the fact that both neutrophils and eosinophils originate from the same haemopoeitic colony forming unit, or there is an actual compensatory increase in number and phagocytic capabilities of eosinophils to correct for the defective neutrophil function[12], evidenced by the presence of eosinophil formazan cells in the NBT test which was also observed earlier by Azmy et al (1977) (unpublished data), although bacterial killing is not a major assignment for eosinophils[1]

The decreased percentage of formazan cells in the NBT dye reduction test in our patients reflects the defective ingestive and oxidative capabilities of the neutrophils. This observation was in accordance with many workers, [3,13] while other workers reported no change in NBT test.[10,14,15,16] This discrepancy could be explained by the variations of population tested or the techniques used.

Defective chemotactic activity of neutrophils in our patients was previously similarly reported,1[1,17,18] while others were not able to demonstrate the same finding.[14,19] Defective chemotaxis in cases with pyogenic skin infections may be due to excessive generation of chemotactic factors in the inflamed skin which might induce chemotactic deactivation or might recruit subpopulation or more responsive cells leaving the less responsive neutrophils in the circulation.[20]

The presence of an inhibitory serum factor t othe neutorphils may result in depressed chemotaxis as suggested recently.[3] The highly significant decrease in IgM among patients especially those with severe recurrent pyogenic skin infection when compared to the control group could be explained by the over consumption of serum IgM as a neutralizing antibody or as a direct mircobicidal agent to compensate for the associated defective neutrophil function. This finding was also supported by the presence of an inverse correlation between chemotaxis and serum IgM levels among some groups of patients.

The relative increase of serum IgA of patients may have its deleterious effects on neutrophil functions. IgA is known to be cytophilic for neutrophils and subsequently suppressing chemotaxis.[11] Also, this increase may be a part of the universal increase of all immunoglobulin subclasses in response to infection, and as serum IgA has no role in host defense, it is not consumed and thus attaining high serum levels.[2]

IgE serum level was markedly high among patients than controls. This finding was noticed in 1980.[21] The authors demonstrated antistaphylococcal immunoglobulin of the IgE subclass in sera of patients with elevated IgE and recurrent pyogenic infections caused by staphylococcus aureus. On the other hand, it was found that sera of patients with high IgE did not impair the chemotactic activity of the control leukocytes,[22] meaning that high serum IgE level is not directly related to the associated defective chemotaxis.

Serum IgG level was significantly low among the group of patients with severe reucrrent pyogenic infection only; which may be due to over consumption as an opsonic and neutralizing antibody used excessively in the almost continuous state of infection, or may be due to decreased production.[24]

The increased serum C4 levels signifies hypoconsumption or hypoactivation of complement which may be due to the presence of low serum levels of IgM, IgG which in turn did not allow antigen-antibody complexes with subsequent activation of the classical complement pathway.

Staph. aureus was the most commonly isolated organism from the lesions of patients in group 1 and group 2 where it was isolated

from 65.4% and 86.6% respectively, this finding was previously repeatedly reported.[4,25,26] The low frequency of Strept. pyogenes in the culture of pus swabs even from lesions known to be caused by streptococci, e.g. ecthyma can be explained by the effect of bacteriocins produced by staph. epidermidis in almost 20% of cases with recurrent pyogenic skin infections emphasizing its potential pathogenic role particularly when the defense mechanisms are defective.[27]

From the above mentioned data we can conclude that the neutrophil chemotaxis, the neutrophil ingestive and oxidative metabolic activities (as measured by NBT test), and the serum IgM levels are the limiting factors in defensive mechanisms against recurrence or chronicity of pyogenic skin infections.

Is defective neutrophil function a cause or a result to the recurrent pyogenic infection? We can assume it is a cause for recurrences and severity of infection evidenced by:-

1. Repeated antibiotic therapies were not effective to control the infection most probably due to defective neutrophil functions.
2. As reported by Solomkin et al[28], depressed neutrophil function precedes the clinical signs of infection by enough time 5.2 +-1.3 days) to predict the occurrence of infection.

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