

The mucosal and systemic immune status for diabetic and non-diabetic Dentoalveolar infected patients

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Abstract:

From a group of 60 patients with dentoalveolar infections among which 10 were diabetic and 10 non-diabetic were elected as test group as well as 10 normal subjects as control group. Six *Staphylococcus aureus* and *Streptococcus anginosus* were diagnosed in the first and second group of the patients the immune status of the patients and control subject were tested by pathogen specific antibody titre, neutrophil NBT reduction phagocytosis and leukocyte inhibition LIF.

Diabetic patients with dentoalveolar infection shows decreased specific antibody titers, subnormal neutrophil NBT phagocytic % as well as non significant LIF % in comparison non diabetic reveal high specific antibody titers against , high neutrophil NBT% and significant LIF% respectively thus diabetic indicate humoral and cellular immune suppression along the den to alveolar infection.

Key word: dentoalveolar , *Staphylococcus aureus* , *Streptococcus anginosus*

Introduction:

Several immune abnormalities have been involved in the immunopathogenesis of the diabetic syndrome (DS). These abnormalities are including direct B-Cell distribution , cytokine influencing B-cell distribution, auto reactive T-Cell & B-Cell distribution as well as the inhibiting insulin receptors on cell surfaces [1,2]. Two types of diabetic syndrome are known, the type insulin dependent mellitus (IDM) and type insulin independent mellitus (IIDM).

In type IDM, however, these abnormalities are islet cell antibodies, insulin antibodies insulin-anti insulin complexes, antigen non-specific complex and insolinome antigen 3 (IA2) [1,2] in the humeral arm of the immune system while, in the cellular arm; auto reactive T-cell causing B-cell distribution that lead to IIDM [2,3]

The immunopathogenesis of type IDM can be briefed as 1) viral

infection or inflammatory stimnli will activate APCS, 2) APC in turn secret mediators that attract lymphocyte to rolling and entry into pancreases tissue; 3) Beta cells either destroyed by direct cytokine or through cytokine secreted by lymphocyte or APCs [2] . In type IIDM the insulin-resistant DM, the diseases effect is caused by interference of antibody binding the insulin receptors on cell surface in which antibody inhibits insulin binding to its receptors leading to insulin resistance with apparent hyperglycemia and ketoaciosis [4] DS as a systemic disease affect pathologic, effects on various organ system of human body including vulnerability to infections and immunosuppression. In the present work attempts were made to test its influences on infected patients.

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Materials and Methods:

1-Patients and Controls;

Ten diabetic and ten non-diabetic dentoalveolar infected patients (1-3) from both sexes were the attendance of the teaching dental clinic during the period of Jan. 2009-Dec. 2009 university of Babylon, they were elected as test group while the Ten of the apparently health normal subjects from both sexes and same age group of the patients were elected as control group from the university staff personals.

2- Bacteriology;

The dentoalveolar materials of the ten diabetic and ten non-diabetic as well as ten normal subjects were swabbed by sterile cotton swab and immersed into 3 mls. sterile normal saline in plane sterile clean tubes, through mixing was done for these samples, then loopful inocula were taken from the swab saline mixture (SSM) and quadrate streaked onto blood agar and nutrient agar plates, and incubated for an overnight period under aerobic conditions. Pure culture were obtained from the primary plate cultures showing the indicating colony morphotypes. The biochemical identification of the pure isolates were done as in [5] and [6].

3- Immunology;

The SSM becomes slightly opaque due to protein and cellular contents. This SSM suspensions were centrifuged at 3000 rpm for five minutes. Supernates were aspirated and tubed into sterile clean tubes and designed as supernate tubes (ST). Three mls of 6% PEG 6000 solutions were added to STs then left at 4°C for 1hr then proceed for immunoglobulin separation as in [7,8] tubes containing SSM deposits designed as deposit tube (DTs). Deposited in DTs were washed ones with sterile saline and resuspended in three mls sterile saline. The deposit washed cell suspensions

were mixed with 3 mls of dextrane and left at 25°C (room temp) for 20 min. the dextrane-leukocyte upper layer was aspirated and tubed into plane tubes, then processed for leukocyte separation as in [9].

Blood with and without anticoagulant in three mls amounts were collected from the patients and controls. [10] standard tube agglutination tests was done on patient sera and SIs with their repeat in antigens [10]. Neutrophil phagocytosis were done by nitroblue tetrazolium reduction percentage [11] leukocyte inhibitory factors was done on mucosal leukocyte and peripheral blood leukocyte [12].

Results:

1-Dentoalveolar infections;

Pure culture and heavy growth of coagulate positive *S.aureus* were noted in six diabetic and six non-diabetic patients with dentoalveolar infections. In addition to four cases of diabetic and for non-diabetic cases of dentoalveolar infections were mounted associated with *S.anginosus*.

2- Cell mediated immunity;

The mucosal and systemic neutrophil phagocytosis for normal control subjects were 9-8 % and 10-8 % respectively. Non-diabetic mucosal and systemic neutrophil phagocytosis in case of *S.aureus* were 43-66 % and 38.5 % NBT %. While in the cases of *S.anginosus* they were 41.5% and 33.25% for mucosal and systemic compartments. In diabetic, however they were in *S.aureus* cases as 27.3% and 28.33% for mucosal and systemic compartments. Likewise, *S.anginosus* diabetic cases were, 41.5 and 33.25% respectively (table 1).

3- Leukocyte Inhibitory Factors (LIF)

In normal control subjects, LIF % were of 0.945 and 0.946 while, in non-diabetic *S.aureus* and *S.anginosus* cases LIF values were ranging between

0.5-0.68% in comparison to the diabetic LIF values were between 0.7 to 0.9 (table 2).

4- Antibody mediated immunity;

The titres of the pathogen specific antibodies at systemic responses; were ranging between 2 to 10 in normal control subjects; and 160 – 200 in diabetic patients as well as 260-265.6 in non-diabetics. While at mucosal compartments they were 32-40 in diabetics and 26-28 in non-diabetics the mucosal globulin concentrations in diabetic ranges from 0.55 to 0.16 mg/ml. While in non-diabetic were ranging from 0.77 to 0.85 mg/ml as compared to control subject with 0.249 mg/ml. The total serum globulin concentrations ranges from 37 to 39 mg/ml in diabetic and 41 to 44 mg/ml in non-diabetic against 36.229 mg/ml for control subjects (table 3).

Table 1: Neutrophil NBT% among diabetic and non-diabetic dentoalveolar patients

Study group	NBT%		
	Pt. No.	Mucosal	Systemic
Diabetic <i>S.aureus</i>	6	27.3	38.33
Diabetic <i>S.anginosus</i>	4	26.25	28.5
Non- Diabetic <i>S.aureus</i>	6	43.66	38.5
Non- Diabetic <i>S.anginosus</i>	4	41.5	33.25
Control subject	10	9.8	10.8

Table 2: Leukocyte inhibitory factors in diabetic & non-diabetic dentoalveolar patients and control subjects.

Study group	Pt. No.	LIF	
		Mucosal	Systemic
Diabetic <i>S.aureus</i> patients	6	0.77	0.77
Streptococcus <i>S.anginosus</i> patients	4	0.76	82.5
Non- Diabetic <i>S.aureus</i>	6	0.535	0.66
Non- Diabetic Streptococcus <i>anginosus</i>	4	0.525	0.62
Normal control subject	10	0.946	0.945

Table 3: The humoral immune parameter for *S.aureus* production in diabetic

	Pt. No.	Mucosa l Ig conc. Mg/ml	Serum total protein conc. Mg/ml	Serum global protein conc. Mg/ml	Mucosa l titre	Syste m titre
Diabetic <i>S.aureus</i> Serum	6	0.55	68.63	37.76	32	160
predominant <i>S.anginosus</i>	4	0.61	70.78	38.75	40	200
Non- Diabetic <i>S.anginosus</i>	6	0.85	72.33	41.82	28	266.6
patient <i>S.anginosus</i>	4	0.77	71.23	43.75	26	260
Control subject	10	0.249	66.43	36.22	2	10

Discussion:

Dentoalveolar infections with *S.aureus* and *S.anginosus* are rare (13,18) thus, *S.aureus* (5,15) and *S.anginosus* (6,17) are being reported in diabetic dentoalveolar infected patients [13,14,15,16,17,18]. The immune status of these patients are being documented. Phagocytic activity through Nitro blue tetrazolium reduction neutrophil phagocytosis was found reduced in diabetic as compared to non-diabetics [14,18,19]. Test for in vitro Leukocyte inhibitory factors both at mucosal and systemic compartments showed that the leukocyte inhibitory cytokine secretion were found inhibited in diabetes as compared to non-diabetic patients, this is quite right since IL₂ and IL₄ and found reduced in diabetic as compared to normal [20,21]. The specific humoral immune functions were checked through the levels of titers of pathogen specific antibodies were lowered in diabetic than in non-diabetic and control subjects. This may be due to either one or more of the followings; 1- humeral fluctuations of insulin 2- impaired antibody producing B lymphocyte functions [20] and 3- Th₁ – Th₂ counter regulation or bias [21,22]. Thus based on these results

(table 1-3), the basic immune factors can be as;

- 1- Reduced neutrophil phagocytic function.
- 2- Reduced LIF cytokine production.
- 3- Reduced levels of pathogen specific antibody titers.

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الحالة المناعية الموضعية والجهازية لمرضى السكري والمرضى من دون السكري المصابين بالتهاب السن والانتفاخ

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الخلاصة :

من مجموعة مؤلفة من 60 مريض بالتهاب السن والسنخ ، اختير عشر مرضى بالسكري وعشرة من دون السكري بوصفها مجموعة دراسة وعشر أسوياء بوصفهم سيطرة . أظهرت نتائج الدراسة بأن ست كانت مخمجة بـ S.aureus وأربع مخمجة بـ S.anginosus لكل من مجموعتي الدراسة وكانت نتائج الزرع سلبية بالنسبة للأسوياء وجرى جمع ودم مع مانع تجلط ومن دون مانع التجلط ودراسة اختبار تثبيط هجرة الخلايا ، اختزال النايتروبولوتترازاليوم وتقدير عيار الضد المتخصص بالمرضى . أظهرت مجموعة السكري اختزال بعيار الضد المتخصص ، واختزال الخاصية البلعمة وتثبيط هجرة خلايا بيض غير معنوي مقارنة من دون السكري كانت ذات عيارات ضد متخصصة عالية وينسب بلعمة عالية وعامل تثبيط هجرة الخلايا معنوي ، وبذلك ظهر بأن السكري يؤثر تأثير مثبت للاستجابة المناعية الخلطية والخلوية.