

# The Immune status of patients with *S. pyogenes* Tonsillitis

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## **Abstract**

Twenty two clinically and culture proven (tonsillar swab culture ) bacterial tonsillitis were diagnosed as *S. pyogenes* tonsillitis patients. Blood with and without anticoagulant were collected from these patients. Serum total and globulin protein concentration, *S. pyogenes* specific antibody titres and leucocyte inhibitory factors (LIF) were performed. Systemic antibody responses were higher than mucosal antibody responses significant (16 cases) and non significant (6 cases). LIF were noted. Thus *S. pyogenes* epitopes stimulate both mucosal and systemic humoral and cellular responses during the tonsillar infections.

## **Introduction**

Tonsils (T) as lymph nodes are nodular aggregates of macrophages and lymphocytes that are layed down onto delicate reticular tissue and reticular cells (Parslow, 2001).

Tonsils are considered as un capsulated lymphoid tissue associated with mucous membranes. Tonsils and the other organized lymphoid tissue of waldeyeris pharyngeal wring are inciuded with the nasal associated lymphoid tissue NALT such as adenoid (Bradtzeag , 1999,Parslow et al,2001).

Food drink and exogenous and the endogenous potential pathogen can be drained by tonsils then finding their way to local lymphatics to the node matrix engulfed by macrophages their antigens processed and presented to T lymphocyte which inturn activate B lymphocyte to grow proliferation and transfered to antibody synthesizing cells where by either insitive produce antibody or migrate into distant effector sites in the mucosal immune cornpartments. Systemic immune response may parall to this mucosal immune response specific to same pathogen(s) (Mims et al,2001,Parslow etal, 2001). In the present work tonsiler mucosal as well as systemic responses to *S. pyogenes* was investigated in tonsillitis patients.

## **Materials and Methods**

Twenty two ,clinically diagnosed male and femal tonsilitis patients were the attendance of Hospital Maternity and Children in

Babylon provinc during oct-2006- march 2007.The patients tonsils were swabed by cotton swabs following standardized techniques ( Crucishank et al 1975).The swab samples were processed for direct and broth pretreated

Primary swab cultures onto blood agar and MacConky agar plates (Shnawa and AL-Amiedi, 2004, Crucishank et al 1975). These swabbed cultures were incubated at 37 C for 24 hrs. The suggestive Streptococcus- like colony morphotypes on primary plate blood agar cultures were purified in pure culture isolates form .These isolates were tested for hemolysis , motility , catalase, oxidase bactracin & optchin sensitivity , and hydrolysis of esculine using convenctional biochemical test ( Macffdin,2000).Blood samples with and without anticoagulants were collected from each patient for separation of sera& agglutination test as well as capillary migration inhibition of leucocytes respectively( Garvey et al 1977). 30% or higher inhibition scored as significant ( Soberg , 1968).Leucocytes were separated from tonsillar swab using dextran separation method using 30% solution (Metcalf et al, 1986) .then used to load capillaries for capillaries migration inhibition test of mucosal leucocytes .The peripheral blood and mucosal leucocytes inhibition factor (LIF) by capillary method (Soberg , 1968). The following equation was used for measuring LIF.

$$\text{LIF} = \frac{\text{Area of migration with sensitizer}}{\text{Area of migration with saline}} \times 100$$

A 30 % or higher inhibition of leucocyte migration was scored as significant. The protein concentrations were determined for sera and mucosal globulins (Bishop et al , 1986 ).

## **Results**

### **I. Infection:**

Direct swab smears showed streptococcus, like bacteria with Neutrophil, Monocytes and lymphocyte infiltration Direct and indirect swab cultures showed pin point colonies occupied two- third or whole of primary plate culture on blood agar plates and not MacConkey with beta haemolytic colonies are catalase ,non motile and oxidase negative bactracin sensitive and optochin sensitive and the hydrolysis of escoline was negative

Thus ,the infection form was of an acute type and monomicrobic .It wase consistant with S. pyogenes.( Table- 1).

## **II. Globulin concentrations**

There were three concentration levels for mucosal globulins were noted among tonsillitis patients. First , low as 0.4- 0.49g/L (three patients), second, moderate levels 0.5- 0.79g/L (13 patients) and high levels 0.8- 1g/L (two patients ).

Likewise, three concentration levels for serum globulin concentration were also noted as low 30- 35g/L (three patients) moderate 36-45g/L (17 patients) and 46-50g/L (two patients) when the patient globulin concentration is low it is for both mucosal and serum and if it is high it is high for both.

## **III. S. pyogenes specific antibodies:**

Both serum and mucosal proteins were biurate positive, well separated by PEG 6% 6000, form colloidal solution in distilled water, homegenous moderately opoque on light pass. Mucosal globulin was resistant to 2ME.

Mucosal S. pyogenes specific antibodies were with titre range of 8-64. that parallels 80- 640. in the systemic titres (Table- 2).

## **IV. Leucocyte Inhibitory factors (LIF):**

Significant LIF were noted with ranges of 0.5-0.68 both in mucosal and systemic in 16 patients. Non significant in either of systemic or mucosal in two patients. Non significant LIF at both of systemic or mucosal in four patients 0.7-0.75. (Table-3).

## **Discussion**

Exogenous and/ or endogenous *S. pyogenes* (Mims et al,2000,Macffadin ,2000). potential pathogens reaching throat are drained into tonsils via lymph (Southgate et al 1997: Brooks et al 2004.). They are phagocytized, their antigens proccssed,then presented either to B or to Th<sub>2</sub> or Th<sub>1</sub> lymphocytes, insitue in tonsiler tissue (Tabel-1) or in distant mucosal effector site (Parslow et.al 2001). *S. pyogenes* (paragraph I) can be of B lymphocyte and/or Th<sub>2</sub> epitopes types will triggar mucosal and systemic (Table 1 )humoral immune responses (Brandatzaege 1994) while other epitopes of *S. pyogenes* can be of Th<sub>1</sub> activator,potential among which Tdth subsets that induce delayed type hypersensivity as indicated by significant LIF result in 16 patients (Table-3). Thus, *S.pyogenes* induce humoral and cellular immune responses both at mucosal and systemic levels (Zoblcr 1998, Soberg,

1968).The non significant LIF results were matched in aged patients(Bloom , 1982; Mims et al ,2000; Shnawa & AL-Amiedi,2004)

On the basis of the results presented in paragraph I, table 1&2. The major immune features tonsillitis patients are

- i. The infection was acute monomicrobial.
- ii. The tonsillitis pathogen is *S. pyogenes*.
- iii. The *S. pyogenes* immunodominant epitopes may be either one or more they can be of B cell, Th<sub>2</sub> or Th<sub>1</sub> dependent.
- iv. *S. pyogenes*. stimulate mucosal as well as systemic , humoral and cellular immune responses.

Table- 1 .*The S. pyogenes* isolate characteristics

Criteria	Result
1-Microscopy & Inflammatory reaction	Gram positive cocci ,in chains. The inflammatory cells are basically as Neutrophil, some were with Monocyte and / or Lymphocyte.
2-Growth	Aerobic
3-Colony -Morphotype	Pin- heald B-hemolytic colonies on blood agar
4-Biochemical and Physiologic tests	
Motility	Non-motile in semi-solid media
Catalase	Negative
Oxidase	Negative
Esculin hydrolysis	Negative
Bacitracin	Sensitive
Optochin	Resistance

Table 2: *S. pyogenes*. Specific antibody response in tonsillitis patients.

Antibody Titre			
No. f Patients	Mucosal (M)	Systemic (S)	S/M ratio
4	8*	80**	10/1
5	16	160	
10	32	320	
3	64	640	

\* 2ME resistant S= systemic

\*\* IgG responses M= mucosal

Table -3 : Leucocyte inhibitory factor in systemic and mucosal leucocytes among tonsillitis patients.

Result		Significance		
LIF Mucosal	Range Systemic	Mucosal	Systemic	Patient Number
0.5-0.68/	0.5-0.69	S	S	16
0.7-0.72/	0.5-0.68	NS	S	1
0.5-0.68/	0.7-0.72	S	NS	1
0.7-0.75/	0.7-0.75	N.S.	N.S.	4

S= significant, NS= Non significant.

## References

- 1- Bishop, M.C.; Dben- Von, J. and Fody, E.P. 1986. Clinical Chemistry, Murray Printing Company, Philadelphia, 180.
- 2- Brooks, G.E.; Butel J.S and Morse, S.A.2004. Medical Microbiology 23<sup>rd</sup> ed. MaGraw. Hill, Boston.
- 3- Brandatzaeg, P. 1994. Handbook of Mucosal Immunology, San Diego, Acadmic press.
- 4- Cruickshank, R.; Duguid, J.P.; Marmoin, B.P. and Swain, R.H.A-1975. Medical Microbiology 12<sup>th</sup> ed. Vol.2. Churchill- Livingstone, London.
- 5- Garvey, J.S., Cremcr, N.E. and Sussdorf,D.H.H press 1977:Methods In Immunology, penjamin Reading.
- 6- Johnstone, A. and Thorpe R. 1982. Immunochemistry In Practice. Black well Scientific Publications, Oxford.
- 7- Mecffadin 2000. Biochemical Identification of Medical Bacteria 3<sup>rd</sup> ed. Lippincott- Williams Wilkin Co- Baltimore.
- 8- McCoy, A.M. and Kenndy E.R. 1969. Autogenous Vaccine therapy in *staphylococci* infections. JAMA .174:35-38.
- 9- Parslow, T.G.; Stites, D.P.; Terr, A.I and Imbodem, J.B. 2001. Medical Immunology 10<sup>th</sup> ed. Lange Medical Publication NewYork.
- 10- Shnawa, I.M.S. And Al-Amiedi, B. H. H. 2004. Immunology of *E. coli* persistent pyuria. Med. J. Bab. 1 (3&4).
- 11- Soberg, M.1968. Invitro Migration inhibiotim of ppheripheral blood leucocyte in delayed type hypersensitivity Acta. Med. Scand. 184:325.
- 12- Southgate, L.; Lockie, C.; Heard, S. and Wood, M. 1997. Infection, Oxford General Practice Series 40. Oxford Medical Publications, London.
- 13- Zabler, R.H. 1998. Antigens, T dependent and independent. In Dalves, P.J. and Roitt, I.M. (eds) Encyclopedia of immunology 2<sup>nd</sup> ed. Vol. I., 214-218. Academic press London.

# الحالة المناعية لالتهاب اللوزتين المتسبب عن **S.pyogenes**

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## الخلاصة

جرى تشخيص 22 حالة التهاب لوز بالموكرات السبحية **S.pyogenes** بالطرق السريرية جمعت عينات دم مع مانع تجلط ومن دونه كما قدر كلوبيلينات المصل ومخاط اللوز و عيار الاضداد المتخصصة واختيار تثبيط هجرة الخلايا البيض **LIF** كان عيار الاضداد البدنيه اعلى من عيارها المخاطي و اظهرت تثبيط هجرة الخلايا البيض نتائج معنوية في 16 حالة وغير معنوي في باقي الحالات وبهذا فان الذرى المستضدية للمكورات السبحية المقيحه تحت الخلايا البائية والتائية اثناء التهاب اللوز

