

THE STUDY OF OPSONIZATION ACTIVITY OF THE PHAGOCYTOSIS PROCESS IN MENINGITIS, SEPSIS, AND RECURRENT INFECTION IN CHILDREN

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ABSTRACT

In this survey, opsonization activity of sera in 110 patients with meningitis, sepsis and recurrent infection; 12 patients after healing; and 50 normal adults has been studied. Opsonization activity was measured by an assay depending on electronic particle counting to measure yeast uptake (alternative pathway activator)³⁶ which was modified in our laboratory.

Three classes of immunoglobulins (IgG, IgM, IgA) and C₃ levels were also studied depending on single radial immunodiffusion by using Behring kit in our patients.

54 pathological sera with defective opsonization showed low C₃ levels, two with lower than normal opsonization activity showed low C₃ levels, 11 defective opsonization cases showed normal C₃ levels, 3 of which were related to diabetic patients.

22 instances of decreased IgG, 14 cases of decreased IgM and 18 cases of decreased IgA were observed in 110 patients.

The opsonization activity, C₃ and immunoglobulin levels in 12 patients have become normal after healing.

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INTRODUCTION

Phagocytosis is one of the first lines of defense against infection. The phagocytic process is composed of four interrelated phases: chemotaxis, opsonization, ingestion and digestion.^{2,4,29,30,50} Most organisms, especially extracellular organisms have surface factors which enable them to resist against phagocytosis.^{48,56} Opsonization is the important process which enhances phagocytosis of these organisms. The function of serum opsonins is to react with microorganisms and to increase hydrophobicity, thereby reducing the charge repulsion between microorganisms and phagocytic cells and make them more susceptible to ingestion by phagocytes.^{24,42} Opsonization of bacteria may occur by at least one of the mechanisms as noted below:

- 1) specific antibody alone (IgG₁, IgG₂, IgG₃, IgG₄).
 - 2) specific antibody (IgG, IgM) acting in concert with complement by activating C₃ via the classic pathway of C₁, C₄ and C₂.⁴²
 - 3) opsonization can be non-specific which is done via activation of alternative pathway and generation of C₃b,^{8,42} fibronectin,^{6,9,13,16,41,49,55} tuftsin,¹¹ C₄b, C₅ and C.R.P.^{35,51}
- C₃b acts via CR₁, CR₃; IgG via FC-receptors on mononuclears, PMNs and eosinophils;^{3,39,60} fibronectin acts via receptors on mononuclears and neutrophils,^{11,41} tuftsin acts via receptors on neutrophils and C₄b acts via CR₁ receptor on phagocytic cells.^{17,22,25}
- Opsonization defects may be primary (genetic)²¹ (Table 1) or secondary after some diseases such as

Phagocytosis Process in Meningitis

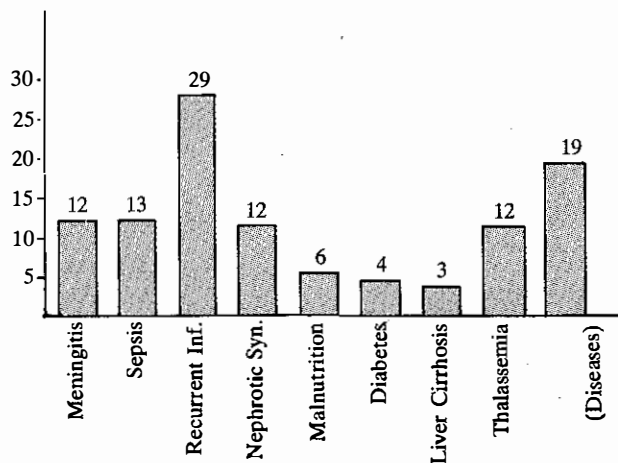


Diagram (1): Number of samples of each disease.

malnutrition, chronic liver diseases,^{23,44,59} diabetes,¹⁵ nephrotic syndrome,^{21,40,43} partial lipodystrophy,²¹ uremia,³² splenectomy,^{1,3,7,10,18,19} septicemia,^{1,34,37,46} immune complex disease,^{21,43,44} excessive immune suppression, bacterial, viral and fungal infections,^{24,27,28,45,52,54,57,58} after some cases such as surgery (cardiopulmonary bypass),^{51,53} burns,^{35,47} trauma,⁵⁵ and after using some drugs and antibiotics⁶¹ or it may occur after inhibition of opsonins or their receptor function. Opsonization defect may occur after inhibition of phagocytic cell function because of existence of inhibitors.

Causes of opsonization defects are low synthesis, excessive loss, excessive catabolism, excessive stimulation of complement system and excessive immunosuppression.

SUBJECTS

In this survey opsonization activity and three classes of immunoglobulin (IgG, IgM, IgA) and C₃ levels of 110 patients (aged 1 month-13 years), 44 female and 66 male, with meningitis (12 cases, 10.91%), sepsis (13

cases, 11.82%), recurrent infection (29 cases, 26.36%), nephrotic syndrome (12 cases, 10.91%), malnutrition, and chronic diarrhea (6 cases, 5.15%), liver cirrhosis (3 cases, 2.73%), diabetes (4 cases, 3.64%), primary immunodeficiency (19 cases, 17.27%), and thalassemia (12 cases, 10.91%) (diagram 1, Table V), and 12 patients with meningitis, sepsis and recurrent infection after healing were studied. Opsonization activity in 50 healthy adults (aged 17-60 years) (26 female, 24 male) were also studied.

MATERIALS

- Balanced salt solution.
 - Hank's
 - RPMI-1640
- Live-baker yeast (*Saccharomyces cerevisiae*) was suspended in 0.85% saline and inactivated at 100°C for 30 min in water bath, after washing for 2-3 times with saline resuspended to 100×10⁶ particles /ml.
- Sera:

Sera were separated from blood as soon as possible and stored at -70°C until analysis.
- Normal PMN were separated from heparinized blood (20 unit heparin for 1 ml blood) by dextran (6%) sedimentation of red cells. After 45 min at room temperature, washed 3-5 times in Hank's and resuspended to 10×10⁶/ml.

METHODS

In this survey we have used ZFCoulter counter with dual size threshold instead of ZB model. By using this system, both yeast particles and phagocytes are counted. Then the opsonization activity was reported as percent uptake of yeast, and also because in diabetic patients at 30 min (the usual time of incubation) more than 90% of phagocytes ingest yeast. Then there will be no difference between normal and diabetic patients. After using different times of incubation, the best time

Table I. Number of sample in each disease

Malnutrition		Nephrotic Synd.		Recurrent Infection		Sepsis		Meningitis		Description
Percent	No.	Percent	No.	Percent	No.	Percent	No.	Percent	No.	
5.15	6	10.91	12	26.36	29	11.82	13	10.91	12	Tests
		Immunodeficiency		Thalassemia		Liver Cirrhosis		Diabetes		Description
Percent	No.	Percent	No.	Percent	No.	Percent	No.	Percent	No.	Tests
100	110	17.27	19	10.91	12	2.73	3	2.64	4	

Table II. Opsonization-defect and C₃ deficiency in 110 patients

Diseases	Opsonization-defect				C ₃ Deficiency			
	Low-Normal		Defect		Low		Low-Normal	
	Number	Percent	Number	Percent	Number	Percent	Number	Percent
Meningitis	1	8.33	6	50	7	58.33	—	—
Septicemia	—	—	10	76.92	9	69.23	—	—
Recurrent-Infection	2	6.90	12	41.38	9	31	2	6.90
Nephrotic Syndrome	—	—	8	66.67	6	50	1	8.33
Malnutrition	—	—	5	83.33	4	66.67	—	—
Diabetes	—	—	4	100	1	25	—	—
Hepatic Cirrhosis	—	—	3	100	3	100	—	—
Thalassemia	1	8.33	4	33.33	5	41.67	—	—
Immunodeficiency	1	5.26	10	52.63	8	42.10	1	5.26

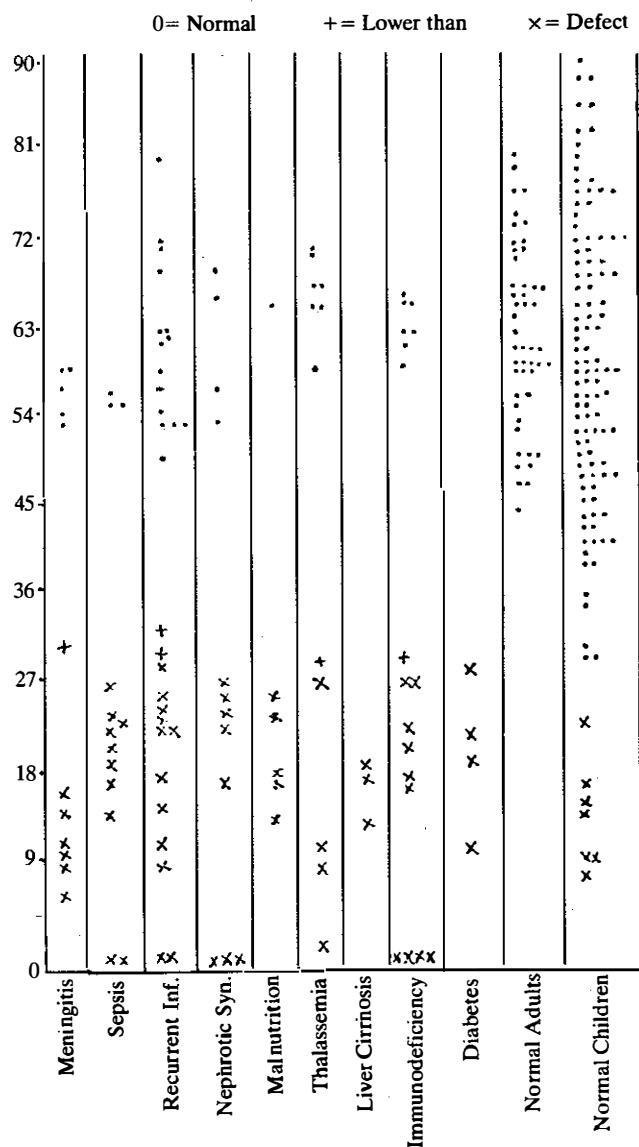


Diagram 2. Opsonization activity results in different diseases. Normal adults and normal children.

was 5 min, thus the defect of these patients was obvious.

C₃ opsonization activity is studied in this method because yeast is an activator of the alternative pathway of complement.²⁰ IgG and C₅ are not effective in this method. Immunoglobulin (IgG, IgM, IgA) and C₃ levels were measured depending on single radial immunodiffusion by using Behring kit in this survey.

RESULTS

51 pathological sera with defective opsonization showed low C₃ levels, two cases of lower than normal opsonization activity showed low C₃ levels, 11 defective opsonization cases showed normal C₃ levels—this case has been reported to be a relatively common immunodeficiency, three of 11 cases of defective opsonization with normal C₃ levels were related to diabetic patients (Table II).

Opsonization activity in 110 patients, 112 normal children, and 50 normal adults is shown in diagram 2.

22 instances of decreased IgG, 14 decreased IgM, and 18 decreased IgA were observed in 110 patients (Table III).

The mean percent uptake activity in 110 patients aged one month-13 years (41 female, 66 male) was 47.2±34.86; 2 S.D. range: 0-80.

The mean percent uptake in 50 healthy adults aged 17-60 years (26 female, 24 male) was 64±18; 2 S.D. range: 46-82.

The mean percent uptake in 112 children aged 11-12 years (47 female, 65 male) was 55±27; 2 S.D. range: 16-83.

The opsonization activity in patients in comparison with normal adult group (T= 11.26, P<0.001) and in comparison with normal children group (T= 7.78, P<0.001) was lower than control group (Table IV, V).

The results in this study have been compared with mean values from opsonization activity in 112 normal

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Table III. Immunoglobulin deficiency in 110 patients

Diseases	Immunoglobulin deficiency					
	IgG		IgM		IgA	
	Number	Percent	Number	Percent	Number	Percent
Meningitis	3	25	1	8.33	2	16.67
Septicemia	—	—	—	—	—	—
Recurrent Infection	3	10.34	1	3.45	2	6.90
Nephrotic Syndrome	5	41.67	2	16.67	1	8.33
Malnutrition	2	33.33	1	16.67	1	16.67
Diabetes	—	—	—	—	—	—
Hepatic Cirrhosis	—	—	—	—	—	—
Thalassemia	1	8.33	1	8.33	1	8.33
Immunodeficiency	8	42.10	8	42.10	11	57.80

Table IV. Comparison between results of 110 patients and normal adults

Number	Cases	Age	Female	Male	Opsonization activity range	Mean \pm 2 SD	T	P
110	Patients	1 Month-13 Years	44	66	0-80	47.2 \pm 34.86	11.26	0.001
50	Normal Adult	17-60 Years	26	24	46-82	64 \pm 18		

Table V. Comparison between results of 110 patients and 112 normal children

Number	Cases	Age	Female	Male	Opsonization activity range	Mean \pm 2 SD	T	P
110	Patients	1 Month-13 Years	44	66	0-80	47.2 \pm 34.86	7.78	0.001
112	Children	11-12 Years	47	65	16-83	55 \pm 27		

children.

Bacteria which were isolated from blood, CSF and infectious sites were as follows: *S. aureus*, Pneumococci, *Haemophilus influenzae* type-b, *E-coli*, Klebsiella, Enterobacter, *Neisseria meningitidis*, *Salmonella paratyphi*.

of about one in 20 (5%) of normal population.^{28,31} Defective opsonization with normal C₃ levels were shown in diabetes; the cause of this defect is the reaction between glucose and opsonins (C₃b, FC of IgG) and their receptors on phagocytes.⁵

DISCUSSION

Based on the results of the present investigation and other studies, opsonization is an important line of defense against extra-cellular organisms.

In this study, lower than normal opsonization activity with low C₃ levels was observed.

Based on other investigations (Kerr, et al., 1983) this is related to fibronectin.³¹

Defective opsonization in individuals with normal C₃ levels were observed also. This case has been shown to be a relatively common immunodeficiency (Soothill and Harvey, 1976; Johnson, 1980 a, b).

This hereditary deficiency occurs with a frequency

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