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DEFECTIVE SERUM OPSONIZATION ACTIVITY IN CHILDREN AGED 6 – 48 MONTHS HAVING ACUTE PURULENT OTITIS MEDIA*

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SUMMARY: Tezcan İ, Yılmaz Y, Öner F, Yel L, Sanal Ö, Ersoy F, Önerci M, Berkel Aİ. (Immunology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Defective serum opsonization activity in children aged 6-48 months having acute purulent otitis media. Trk J Pediatr 1997; 39: 453-457.

Serum opsonization of yeast (Saccharomyces) was investigated in 51 patients whose ages were between six and 48 months (median 15 months) with acute purulent otitis media and in an age-matched control group (median 13 months). Opsonization was assessed by measuring yeast particle uptake in an assay based on an electronic count of the unphagocytosed particles in serum by polymorphonuclear leukocytes. Despite normal levels of CH50 and serum immunoglobulins, a defective opsonization was determined in 13.7 percent of the patients (7 in 51). The corresponding figure was 2.9 percent in 103 healthy controls ($p < 0.001$). On the other hand, 218 percent (5 in 23) of the children having a history of recurrent purulent otitis media showed defective opsonization ($p < 0.001$). Previously, the presence of an opsonization defect has been linked to low levels of mannan binding lectin (MBL), a calcium dependent serum lectin that acts as an opsonin. Therefore, our findings indirectly support the idea that MBL has an important role as host defense, particularly in the earlier period of life when the antibody repertoire is restricted. *Key words: common opsonic defect, acute otitis media, mannan binding lectin.*

Acute otitis media (AOM) is one of the most common infectious diseases of childhood[1,2]. The peak incidence of AOM occurs in the second half of the first year of life, with a second, lower peak, between the ages of four and five years. Most of the children have their first episode of AOM in their first two years of life. Opsonophagocytosis is the key immune mechanism against encapsulated bacteria (S. pneumonia, H. influenzae, etc.) the leading cause of AOM[3]. During the early years of life, the antibody repertoire is restricted. Members of the

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collectin family, for instance mannan binding lectin (MBL), may play a critical role in opsonization of sugar-containing bacteria[4-6]. Super et al.[5] showed that common opsonic defect is linked with low serum levels of MBL.

Evaluation of the host defense and risk factors, such as bottle feeding, parental smoking, and dysfunction of the eustachian tube, are of utmost importance for the treatment of both AOM and its possible complications. In this study, we investigated serum opsonization activity in 51 children with AOM to determine whether common opsonic defect is an important risk factor for acute purulent otitis media when the antibody repertoire is restricted.

Material and Methods

The study group consisted of 51 patients aged between six and 48 months (median 15 months, 28 male, 23 female) who were diagnosed as AOM without apparent underlying immunodeficiency in Hacettepe University Children's Hospital during the period December 1993-May 1994.

The diagnostic criteria of AOM were fever, earache, purulent discharge in the external ear canal, and perforation and hyperemia of the eardrum. The patients having three or more attacks of acute purulent otitis media during the six months prior to admission were defined as the recurrent otitis media group. (ROMG, n: 23).

Each case was examined by an ear, nose and throat (ENT) specialist to exclude from the study the cases with hypertrophic adenoids.

The study also consisted of a control group of 103 healthy children aged between six and 46 months (median 13 months, 57 male, 46 female).

Serum samples were obtained two to three weeks after the acute illness and stored at -30 °C until the analysis. Quantitative serum immunoglobulins, CH50, and IgG subclasses of the patients were studied by conventional methods.

Measurement of serum opsonic activity: An objective semi-quantitative method for killed baker's yeast (S. cerevisiae) opsonization by normal neutrophils was used[7]. Briefly, 10 µl patient's serum, 50 µl 0.9 percent NaCl, 50 µl yeast particles (108/ml) and 100 µl healthy neutrophils (PMN) (1 x 10^7/ml) were mixed together in a plastic tube and incubated at 37 °C for 30 minutes on a mixer. In controls, 50 µl yeast particles made up to the same reaction volume of only saline were similarly incubated. After completion of the incubation, the reaction mixture was diluted 1:500 in Isoton (Coulter Electronics Ltd.). The number of yeast particles left unphagocytosed were counted in a Coulter Counter, set to count particles 3-5 µm. Result are expressed as percentage uptake of yeast by normal PMNs and are calculated as follows. Percentage uptake: The number of yeast particles added to the reaction (A)-the number of yeast particles left unphagocytosed: A x 100.

The deficient serum was defined as the value less than two standard deviations (-2SD) from the mean measured in the healthy control group.

Student's t and Mann-Whitney U tests were used for the statistical analysis; p value below 0.05 was considered significant.

Results

The percentage of yeast opsonization in the healthy controls was 60.9 ± 13.6 percent (Fig. 1). The deficiency level was calculated as less than 33.5 percent. The percentages of defective opsonization in the healthy group and the patient group were 2.9 percent (3 in 103) and 13.7 percent (7 in 51), respectively ($p < 0.001$) (Fig. 2). Among the cases with recurrent otitis media (ROMG), the percentage of defective opsonization was found to be much higher (21.8%, 5 in 23) in comparison to the controls ($p < 0.001$).

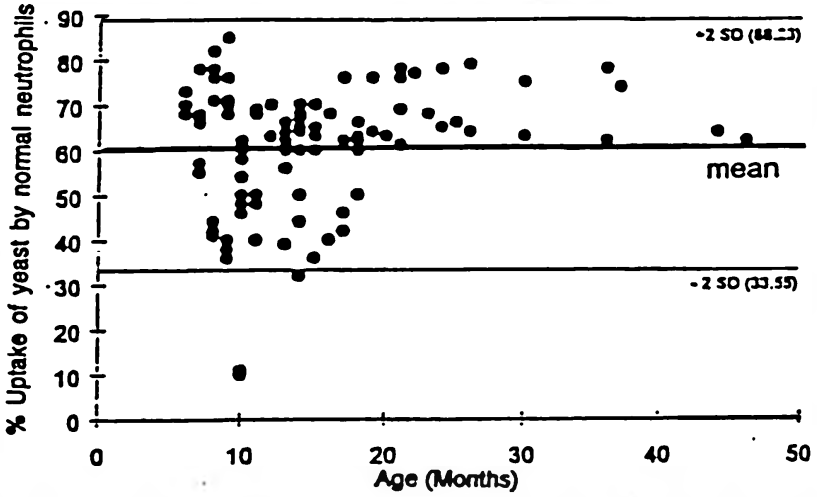
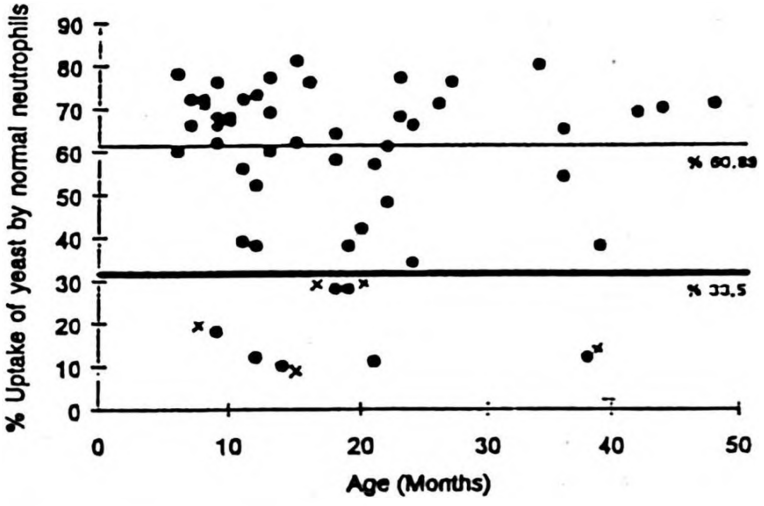


Fig. 1: The distribution of opsonization activity in healthy children.



* The cases with defective opsonization activity from recurrent otitis media group.

Fig. 2: The distribution of opsonization activity in children with acute otitis media.

Other immunological tests (serum immunoglobulins, IgG subclasses and CH50) of the patients were within normal ranges.

Discussion

Miller et al.[8] first defined the failure of serum to opsonize baker's yeast for phagocytosis by normal neutrophils in an infant with severe recurrent infections, diarrhea and failure to thrive. The defect was also shown in a group of children with chronic diarrhea[9]. In 1983, Richardson et al.[10] reported that 26 healthy term babies with defective yeast opsonization activity had a tendency to develop acute otitis media, gastroenteritis and atopy during a one year follow-up period. The defect occurs in five to eight percent of the normal population[6, 11]. In our study, 2.9 percent of the healthy subjects were shown to have defective opsonization activity. The defect was found to be significantly higher in both AOM and ROM groups, when compared to the healthy age-matched controls (13.7 and 21.8% vs 2.9%, respectively). Since all patients with defective opsonization had a history of three to 15 attacks of otitis media, our results may suggest that common opsonic defect could be considered as a contributing factor for the recurrence of acute otitis media in children under the age of four years.

In 1989, Super et al.[5] reported that common opsonic defect is linked with low serum levels of mannan binding lectin (MBL), which is a member of the collectin family of proteins with a collagenous region and a lectin domain. It has various features, including binding a wide range of substrates with multiple carbohydrate binding sites, acting as an opsonin by coating sugar-rich microbial surfaces and also initiating activation of the complement system[12]. It is likely to be a very important component in the innate immune system, particularly in the early steps of inflammation.

Recently, Garred et al.[13] reported that a higher frequency of homozygotes for abnormal MBL alleles associated with a low serum MBL level was observed in 228 unrelated patients suspected of non-HIV related immunodeficiencies, when compared to homozygosity for abnormal alleles in healthy controls (8.3 vs 0.8 percent). In a study conducted to determine MBL levels in plasma of children with recurrent otitis media, the same authors reported no difference in MBL levels in the healthy and diseased children[14]. However, it should be noted that the median age of the patient group was a rather late age of 51.5 months, at which time many defense mechanisms of the immune system have already been developed.

In a recent report, Summerfield et al.[15] showed that the prevalence of MBL gene mutations in children admitted to the hospital due to various infections was about twice that in children without infection. Mutations in the MBL gene which cause a common opsonic defect are associated with the infections. We believe that prospective controlled studies conducted on larger patient populations with various infections will contribute significantly to a better understanding of the role of abnormal alleles of MBL in the distinct susceptibility of children to various types of infections, including AOM.

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