

# Cerebrospinal fluid polymorphonuclear leukocyte elastase levels in diagnosis of childhood meningitis

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*This study includes 40 children with meningitis and 20 controls. In their cerebrospinal fluid samples, alpha-1-proteinase inhibitor complex of elastase was determined by enzyme immunoassay method. Statistical evaluation of the results showed that there was a significant difference in elastase between the patients and controls, being higher in the former ( $p<0.001$ ). Similarly, there was a close relationship between elastase level in cerebrospinal fluid and CSF polymorphonuclear leucocyte count in the patients ( $r=0.700$ ,  $p<0.001$ ). The sensitivity and specificity of elastase test, which were 100 % and 75 %, respectively, showed the test to be of diagnostic value in meningitis, especially of bacterial type. [Turk J Med Res 1994;12(2):70-72]*

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Meningitis is an urgent medical status. The morbidity and mortality rates are closely dependent on the immediate diagnosis and treatment. Within 30 minutes after physical examination, one should establish the diagnosis and begin therapy (1). It is necessary to analyse the cerebrospinal fluid (CSF) sample in order to clearly diagnose, to make correct classification and to establish the differential diagnosis in a patient with meningitis. One may not always obtain typical CSF findings in early stage of the disease in children and in those receiving antibiotics. On the other hand, traumatized CSF samples are not convenient for examination of cell morphology and chemical constituents. Since cultured CSF samples give result after a long duration, reliable laboratory tests of short duration are needed in order to make diagnosis as soon as possible.

Elastase is an enzyme localized in azurophil granules of human polymorphonuclear leucocytes (PMN) and is a lysosomal protease. The enzyme is released into the extracellular space during phagocytosis and inactivated by binding to its inhibitors (2-6). In the diagnosis of systemic and local bacterial

infections, plasma PMN-elastase has been found to be helpful (5). It is also known that the PMN elastase activity is increased in other body fluids such as synovial (4), pleural (3), cerebrospinal (6,7) fluids in acute bacterial infections. The complex of elastase-alpha-1-proteinase inhibitor (E-alpha-1-PI) in plasma increases in newborn sepsis and meningitis (2,6-8). Similarly, the patients with bacterial meningitis (BM) have high levels of E-alpha-1-PI complex (6,7) and PMN-elastase in their CSFs. This study was intended to investigate whether CSF PMN-elastase levels can be used in differential diagnosis of meningitis.

## MATERIALS AND METHODS

The present study includes 40 patients (age range: 1 month - 11 years) with meningitis and 20 without meningitis for whom lumbar puncture (LP) was made for other purposes (age range: 1 month - 10 years). The diagnosis was made on the basis of meningeal irritation and abnormal CSF findings. Patients with bacterial meningitis were identified by the following criteria. All patients had positive results of CSF culture, direct microscopy, and gram stained smears,  $>10$  WBC/mm<sup>3</sup> CSF, and elevated CSF protein concentrations  $>20$  mg/dL. The criteria for aseptic meningitis were  $>10$  WBC/mm<sup>3</sup> CSF, negative CSF bacterial cultures, a negative tuberculin skin test result, and clinical symptoms of meningitis. The diagnosis of tuberculous meningitis was ascertained by culture of Mycobac-

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**Table 1.** The statistical evaluation of the results of the patients and controls.

Parameters	Patients (n=40) X±SD	Bact.men. (n=35) X±SD	Controls (n=20) X±SD	P
CSF protein (mg/dL)	69±55 (24-246)	72±62 (28-261)	34±25 (12-134)	<0.001
CSF PMN count (/mm <sup>3</sup> )	556±676 (20-2400)	648±693 (20-2400)	5±11 (0-30)	<0.001
CSF PMN-elastase (M9/L)	315±192 (23-836)	335±191 (60-836)	41±27 (10-105)	<0.001

terium tuberculosis from the CSF, positive tuberculin skin test result, and clinical symptoms of meningitis.

Within 12 h after hospitalization, CSF samples for biochemical analyses were centrifuged at 4000 rpm for minutes (+4C), since PMNs are destroyed within 90 min. after sampling (9) and elastase is released from the cells (2). The resultant supernatant was stored at -20°C up to the day of analyses. In CSF samples the routine analyses were made for glucose, chloride, and protein. PMN-elastase levels were determined by enzyme immunoassay kit (PMN-Elastase, IMAC, Merck) (10). In the principle of the test, specific antibodies to human PMN-elastase bind covalently to peroxidase, resulting in antibody conjugate. This conjugate binds PMN-elastase in the sample, giving rise to antigen-antibody complex, which shows an enzymatic activity caused by peroxidase as follows:



The resultant product, quinonimine, is equimolar to PMN-elastase. The absorbance of quinonimine is measured at 500 nm. In this study, Bechman Clinic Analyzer (Model 42) was used for the enzyme level determination. Together with the samples, a series of standard working was made and the results were calculated as ng/L from standard curve. Student's t-test was used for statistical analysis. The specificity and sensitivity of PMN-elastase analysis for meningitis were calculated.

## RESULTS

Of 40 patients, 35 were with bacterial meningitis, 3 aseptic meningitis, and 2 tuberculous meningitis. The overall results of the patients and controls are shown in Table 1. All the patients had higher CSF protein, CSF PMN count, and CSF PMN-elastase than the control group ( $p < 0.001$  for all parameters). When one considered the bacterial meningitis group, higher levels of PMN count and PMN-elastase were seen than those of controls ( $P < 0.001$  for both). Correlation analysis was made in the patients between PMN

count and PMN-elastase and a statistically significant correlation was found ( $P < 0.001$ ,  $r = 0.700$ ) (Figure 1). There was also a significant correlation in the patients between CSF protein and PMN-elastase ( $p < 0.01$ ,  $r = 0.430$ ). Sensitivity and specificity of CSF PMN-elastase for bacterial meningitis were found 75% and 100%, respectively, when PMN-elastase value over 41 fig/L, the mean value of PMN-elastase of control group, was considered as positive.

## DISCUSSION

It is well known that elastase localized in azurophilic granules of PMNs is released into extracellular space during phagocytosis, showing a measure of bacterial and leucocyte action (3,5). The previous studies (8) show that there is a significant correlation between PMN activity and PMN elastase levels in plasma in systemic and local bacterial infections. The present study tries to document a possible correlation between PMN-elastase levels and PMN action in CSF of patients with meningitis. For this purpose, E-alpha-1-PI complex was determined in CSF samples by enzyme immunoassay method as done by Speer et al (6,8) and Stoffler et al (7). Speer et al reported E-alpha-1-PI complex concentrations in two different studies, which showed increased CSF E-alpha-1-PI levels in bacterial meningitis when compared with control group ( $P < 0.001$

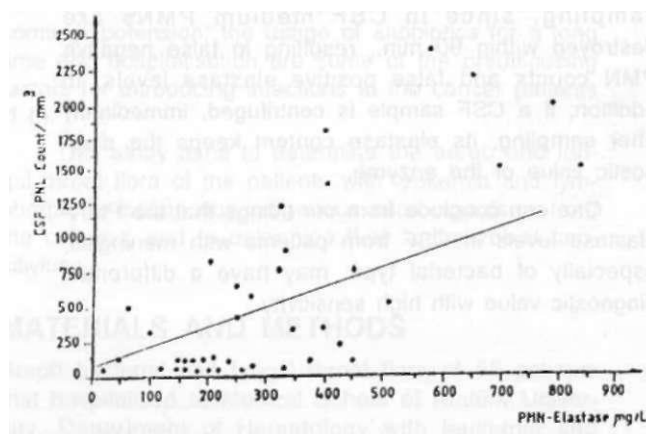


Figure 1. The correlation analysis of PMN count and PMN-elastase in CSF of patients with meningitis ( $n = 40$ ,  $r = 0.700$ ,  $P < 0.001$ )

for both studies). Our results are consistent with these two studies. Stoffler et al reported similar findings.

When PMN-elastase levels in bacterial meningitis are evaluated, one should be alert in the subject of whether the patients with bacterial meningitis have received antibiotic therapy during sampling, since antibiotic usage in bacterial meningitis decreases PMN-elastase levels as reported by Stoffler et al (7). In order to evaluate the effect of antibiotic usage on PMN-elastase in CSF, enzyme levels determination was made in CSF samples of 11 patients with bacterial meningitis who were receiving antibiotic therapy before hospitalization. The obtained levels (115+65 ug/L) were higher than those of controls but lower than those of the patients receiving no antibiotic before hospitalization. Thus, these 11 patients were excluded from the patients group.

Each PMN leucocyte contains approximately 3 picograms of elastase (3). Accordingly, as PMN leucocyte count increases, elastase levels increase in CSF. On the other hand, the presence of elastase inhibitor complex in CSF is not associated with the diffusion of the complex from plasma into spinal fluid, since this complex is formed in CSF itself (6), which is supported by the fact that no correlation is found between PMN count and elastase levels (6,8). Speer et al found no correlation between PMN count and elastase levels, while our study shows the reverse. We can explain why this is the case. For release of elastase PMN must be stimulated for phagocytic activity and this results in elevated levels in CSF. In early stages, there may be increased PMNs and low levels of elastase in CSF.

Five samples of control group were traumatized. That when they were separately evaluated, normal levels of elastase levels were found, suggested no effect of blood mixing into the CSF on PMN-elastase. On the other hand, CSF should be evaluated with respect to cell morphology immediately after sampling, since in CSF medium PMNs are destroyed within 90 min., resulting in false negative PMN counts and false positive elastase levels. In addition, if a CSF sample is centrifuged, immediately after sampling, its elastase content keeps the diagnostic value of the enzyme.

One can conclude from our findings that the PMN-elastase levels in CSF from patients with meningitis, especially of bacterial type, may have a differential diagnostic value with high sensitivity.

Çocukluk çağı menenjitlerinin tanısında beyin omurilik sıvısı polimorfonüveli lökosit elastaz düzeyleri

*Bu çalışmaya menenjitli 40 ve kontrol grubu olarak da 20 çocuk alınmıştır. Beyin omurilik sıvısı örneklerinde, elastaz alfa-1 proteinaz inhibitör kompleksini enzim immunoassay metodu ile tayin edildi. Sonuçların istatistiksel değerlendirilmesinde hasta grubunda elastaz seviyesinin daha fazla olduğu gözlemlendi (p<0.001). Benzer olarak, hastalarda beyin omurilik sıvısı elastaz düzeyi ile BOS polimorf nüveli lökosit sayımı arasında yakın bir ilişki vardı (r=0.700, p<0.001). Elastaz testinin sensitivitesinin % 100 ve spesifitesinin %75 olması, özellikle bakteriyel menenjitlerinde tanısız önemi olduğunu göstermektedir. [Turk J Med Res 1994; 12(2): 70-72]*

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