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IMMUNOCHEMICAL IDENTIFICATION OF *Streptococcus pneumoniae* 40 KDa ANTIGEN IN CEREBROSPINAL FLUID AND SERUM OF *Streptococcus pneumoniae* INFECTED PATIENTS

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ABSTRACT

Bacterial meningitis is a major cause of death and disability in children. *Streptococcus pneumoniae* is one of the common causative agents of bacterial meningitis in children. The diagnosis requires an analysis of cerebrospinal fluid (CSF), this requires a lumbar puncture to confirm the presence of inflammatory cells in the cerebrospinal fluid, to identify the infecting pathogen, and to guide antimicrobial therapy**.** We have identified the 40 kDa protein from *S. pneumoniae* in CSF and Serum with no significant differences between the antigen in serum and CSF and we used Enzyme Linked Immunosorbent Assay (ELISA) in the detection of soluble pneumococcal antigen in serum and CSF samples. So; it is possible to use serum samples in the detection of the *S. pneumoniae* antigen instead of CSF samples.

Keywords: *Streptococcus pneumoniae,* antigen, 40-kDa

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INTRODUCTION

Meningitis is an inflammation of the protective membranes covering the central nervous system, known collectively as the meninges which is typically caused by infection with viruses, bacteria, or other microorganisms. Bacterial meningitis is one of the most severe diseases in Sub-Saharan Africa; more than one million cases are estimated per year, with mortality and life-long sequelae occurring in 50% of these cases (Siga´uque *et al*., 2008). The most common causes of bacterial meningitis after the neonatal period are *Streptococcus pneumoniae* (or pneumococcus), *Haemophilus influenzae* and *Neisseria meningitis* (or meningococcus) (Saez-Llorens and Cracken, 2003; Youssef *et al*., 2004). *S. pneumoniae* are responsible for 80% of cases of meningitis disease in Children and adults, resulting in approximately risk in 50% mostly in developing countries (Beek *et al.*, 2006). *S. pneumoniae* have different virulence factors on its cell surface.

These virulence factors help during infection (Todar, 2003) such as polysaccharide capsule, cell wall, pili and surface proteins (Gillespie and Balakrishnan, 2000; Meli *et al* ., 2002). In the early phases of acute bacterial and viral meningitis, signs and symptoms are often non-specific and it is not always possible to make a differential diagnosis on the basis of routine examination of cerebrospinal fluid (CSF). There are many techniques proposed to diagnose *S. pneumoniae* infection based on the detection of the bacteria including culture, microscopic examination, and immunological

methods that considered as the useful and sensitive tests for diagnosis of *S. pneumoniae* infection. Beside that, gel electrophoresis (SDS-PAGE) technique could be used in the detection of *S. pneumoniae* on the basis of protein profiles, and the Enzyme Linked Immunosorbent Assay (ELISA) in the detection of soluble pneumococcal antigen in sputum, serum, CSF and urine samples (Farrington and Rubenstein, 1991).

So, the aim of the present study is the identification of *S. pneumoniae* antigen in serum and cerebrospinal fluid of meningitis patients using immunochemical and biochemical techniques.

MATERIALS AND METHODS

Serum and cerebrospinal fluid samples

Blood and CSF samples were obtained from 173 Egyptian individuals admitted at Abbassia Fever Hospital, Cairo, Egypt. The samples were clinically diagnosed to have meningitis. They were (111 males and 62 females, aged 3 months – 80 yr, mean age was 20.02 ± 18.59). The meningitis patients clinically classified into 4 groups: Encephalitis; Aseptic; TB- meningitis and Meningococcal as shown in Table 1.

Table 1: Demographic data of Meningitis patients with different manifestations:

Pathological status	Number	Male	Female	Mean age	Median
Encephalitis	90	54	36	20.2	17
TB – meningitis	29	17	12	21	24
Aseptic meningitis	14	10	4	12.4	3
Meningococcal meningitis	40	30	10	21.9	17
Total	173	111	62	20.2	17

Sodium Dodecyle Sulfate −Polyacrylamide Gel Electrophoresis (SDS-PAGE) and gel electroelution

50 µl of infected and non-Infected serum samples was mixed (V/V) with sample buffer. The sample was then boiled in water bath for 3 minutes to ensure complete denaturation of sample protein. The samples were then applied analytical SDS-PAGE, at 20µl/lane, using 12 % polyacrylamide (Laemmli, 1970). Prestained standard molecular weight marker (BioRad, CA, USA) were run in parallel. Electrophoresis was carried out with constant volt at 200 V (Hoefer, USA). The run was terminated when the bromophenol blue marker reaches to the bottom of the gel (45 minutes). The separated proteins on polyacrylamide gel were stained with Coomassie blue R-250 (Andrews, 1986). Coomassie stain gives a linear response up to 20 mg/cm. However, the relationship between stain density and protein concentration varies for each protein (Deutscher, 1990). The electrophoresed gel was soacked in excess of staining solution for one hour with constant shaking.The gel was rinsed with distilled water,then the gel was destained with excess amount of destaining solution for several times with constant shaking until the excess stain was satisfactory removed .

Immunoblotting technique (Western immunoblotting):

Protein electroblotting:

Samples separataed on SDS-PAGE were electrotransformed onto nitrocellulose (NC) membrane in a protein transfer unit (Towbin *et al.*, 1979). The gel, nitrocellulose sheets, sponge sheets and Whattman filter papers were equilibrated for 15 minutes in transfer buffer (86.4 gm glycine (ADWIC) and 18 gm trisma base (Sigma) were dissolved in 4800 ml deionized water, pH 8.3). The blotting sandwich was assembled within the blotting cassette. The cassette was inserted in to blotting (Transfer) buffer and the power supply was connected, as the cathode should be on the gel side. The blotting was carried out with constant voltage of 60 V for 2 hours at 4°C

Immunostaining for nitrocellulose blots using specific anti *S. pneumoniae* antibody:

Nitrocellulose (NC) filter was blocked in blocking buffer (0.3 gm Bovine serum albumin (BSA) (Sigma) were dissolved in 10 ml TBS pH 7.4) for one hour at room temperature.

The NC was washed in TBS (Trisma base, 3.027 gm; Sodium chloride, 5.844gm; Distilled water, 400 ml, pH 7.4) three times for 5 minutes each. The NC was then inserted in sufficient volume of primary antibody (Specific anti- *s pneumoniae* IgG antibody was prepared in blocking buffer (1 %BSA in TBS) in a dilution 1:100) with constant shacking overnight at room temperature. The NC was washed in TBS three times, 15 minutes each. The NC was incubated with secondary antibody (Anti-rabbit IgG alkaline phosphatase conjugate (Sigma) with dilution of 1:500 in TBS with gentle shacking for three hours at room temperature followed by washing in TBS three times 15 minutes each. The blots were visualized by incubating the NC filter in substrate solution Alkaline phosphatase substrate (BCIP/NBT, It is composed of 5-Bromo-4-Chloro-3- Indolyl phosphate (BCIP), (Sigma) and Nitro blue tetrazolium (NBT), system pH 9.6). Then, the reaction was stopped by distilled water, then dried in air and kept in dark. The molecular weight of the protein reactive band was determined according to the method of Chrambach (1985).

Enzyme Linked Immuno-Sorbent Assay (ELISA):

Polystyrene microtitre plates were coated with 50µl/well of diluted serum samples in coating buffer (0.303 gm sodium carbonate (Na_2CO_3), and 0.588 gm sodium bicarbonate ($NaHCO_3$) were dissolved in 100 ml distilled water, then the pH was adjusted to 9.6). The plates were incubated overnight at 4° C. The plates were washed 5 times with 0.05 % (V/V) PBS-T20 (pH 7.2). 200 µl/well of 0.4 % (W/V) BSA in phosphate buffer saline (pH 7.2) (blocking buffer, 0.04 gm bovine serum albumin was dissolved in 10 ml phosphate buffer saline pH 7.2 were added, then, the plates incubated for 1 hour at room temperature. The plates were washed for 5 times (5 minutes each) and stored at 4° C or used immediately. 50 µl/well of primary antibody (Specific anti-*streptococcus pneumoniae* IgG antibody were diluted 1:75 in PBS-T20) was added, and then incubated at 37°C for 2 hours. After washing (5 times), 50 µl/well of secondary antibody (Anti-rabbit IgG alkaline phosphatase (Whole molecule, Sigma) conjugate was diluted 1:350 in PBS-T20 containing 0.2 % BSA) was added, and then incubated at 37° C for 1 hour. After washing,

excess conjugate was removed, and the amount of coupled conjugate was determined by incubation with substrate solution (One mg of p-Nitrophenyl phosphate (Sigma) was dissolved in 1 ml of prepared substrate buffer (glycine buffer) 30 minutes at 37° C. The reaction stopped by addition of 25 µl/well of stopping solution (3 M NaOH). The absorbance was read at 490 nm using ELISA reader. The cutoff level of ELISA above or below which the tested sample is considered positive or negative was calculated as the mean ELISA of serum samples from normal individuals + 3 standard deviation.

Statistical Analyses

All statistical analyses were done by a statistical software package "SPSS 12.0 for windows, SPSS Inc.). The levels of markers were analyzed by ANOVA and the paired *t* test. P values at a two-sided $P < 0.001$ were considered statistically significant. Data were expressed as arithmetic mean ± standard deviation (X ± SD). Spearman correlation test (r) was also used to investigate the relation between each two variables among each group. The result of the t-values was then checked on student's-t-table to find out the significance level (P value). The four conventional probability levels signifying the variable grades of statistical significance are indicated after (Miller and Knapp, 1992).

$P > 0.05$ considered not significant.
$P < 0.05$ considered significant.
$P < 0.01$ considered highly significant.
$P < 0.001$ considered very highly significant.
$P < 0.0001$ considered extremely significant.

RESULTS

The specific polypeptide bands of *Streptococcus pneumoniae* antigen from the selected serum and CSF samples of infected and non-infected patients with meningitis were resolved on the discontinuous high – resolution 12 % one dimensional (SDS- PAGE) under reducing conditions and staining with coomassie blue. The coomassie blue stained separated polypeptides have a wide range of molecular weights ranged from 215 kDa to 18.3 kDa.

The separated proteins were electrophoretically transferred to nitrocellulose (NC) paper. Anti-pneumococcal antibody was used as a primary antibody and anti – rabbit IgG alkaline phosphatase was used as a conjugate (secondary antibody labeled enzyme). The BCIP/NBT system was used as enzyme substrate. An intense sharp band of *S. pneumoniae* antigen was observed in both serum and CSF samples of infected patients but no reaction with serum or CSF samples of non – infected was observed.

The molecular weight of reactive epitope for *S. pneumoniae* antigen in serum and CSF samples of infected and non–infected patients, and linear calibration represents a relation between the molecular weight of protein standards mixture and their flow rates on SDS- PAGE was constructed. The flow rates of the reactive band was calculated and its molecular weight was determined from the liner calibration. The molecular size of the reactive band was the same in both serum and CSF at 40-kDa as shown in Table 2.

The expression of pneumococcal antigen in serum and CSF was detected by using the ELISA technique. The cutoff level of ELISA above or below which the tested sample is considered positive or negative was calculated as the mean ELISA optical densities from healthy volunteers ± 3 standard deviation. It was set at 0.35 and 0.30 for Serum and CSF, respectively**.**

The optical density at 490 nm of 173 serum and CSF samples were tested for pneumococcal antigen using ELISA technique. The pneumococcal antigen was detected in 115 (66%) of 173 CSF samples tested cases and 58 (33.5 %) of CSF samples are negative pneumococcal antigen. Furthermore, the pneumococcal antigen was detected in 91 (52 %) of 173 serum samples tested cases and 82 (47 %) of CSF samples are negative pneumococcal antigen**.**

Table 2: R_f values of unknown antigen and of standard proteins mixture.

Molecular weight	Log Molecular weight	*Relative mobilties (R_f)
205	2.33	0.10
116	2.07	0.12
97	1.92	0.16
66	1.77	0.20
45	1.59	0.40
29	1.44	0.50
40 kDa	1.6	0.32

*** R_f =Migrated distance of protein / Migrated distance of dye**

No significant difference (P > 0.05) between detection rate of pneumococcal antigen in serum (non invasive sampling method) and CSF samples (invasive sampling method) as shown in Figure 1. A significant correlation (r = 0.263 & p = 0.0001) was found in levels of pneumococcal antigen between serum and CSF of total meningitis patients.

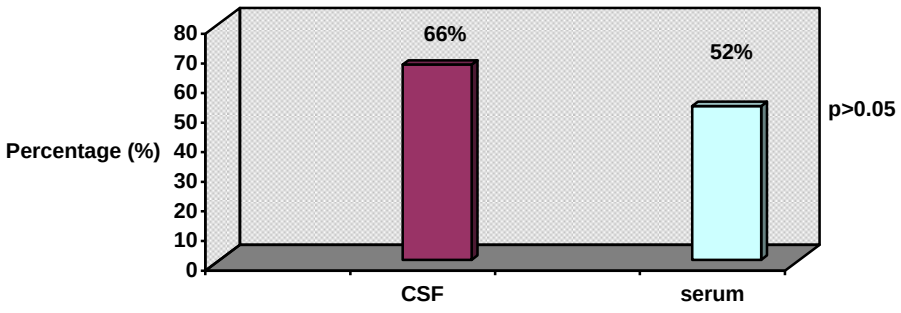

Figure 1: Percentage of positive pneumococcal antigen in 173 CSF and serum samples using ELISA .

The detection rates of pneumococcal antigen in serum and CSF in the different manifestations of meningitis patients and normal controls are shown in Table 3. and Figure 2. The pneumococcal antigen was detected in CSF of 66 % of total meningitis patients , 70 % in encephalitis, 48 % in TB-meningitis, 64 % in aseptic and 72 % in meningococcal patients, while, the pneumococcal antigen was detected in sera of 52 % of total meningitis patients; 54 % in encephalitis, 34 % in TB-meningitis, 50 % in aseptic and 62 % in meningococcal patients Figure 2. The highest detection rate of pneumococcal antigen was shown in meningococcal patients in case of serum and CSF.

Table 3: Detection rates of pneumococcal antigen in sera and CSF of meningitis patients with different manifestations using ELISA.

Pathological status	Pneumococcal Ag in serum		Pneumococcal Ag in CSF	
	% (P/T)	% (N / T)	% (P/T)	% (N / T)
Controls	0 (0/2)	100 (20/20)	0(0/20)	100(20/20)
Encephalitis	54(49/90)	45.5(41/90)	70(63/90)	30(27/90)
TB- meningitis	34(10/29)	65.5(19/29)	48(14/29)	52(15/29)
Aseptic meningitis	50(7/14)	50(7/14)	64(9/14)	36(5/14)
Meningococcal meningiti	62(25/40)	37.5(15/40)	72(29/40)	27.5(11/40)
Total	52(91/173)	47(82/173)	66(115/173)	33.5(85/173

Abbreviation used in the table:
P: number of positive; N: number of negative; T: Total number of patients.

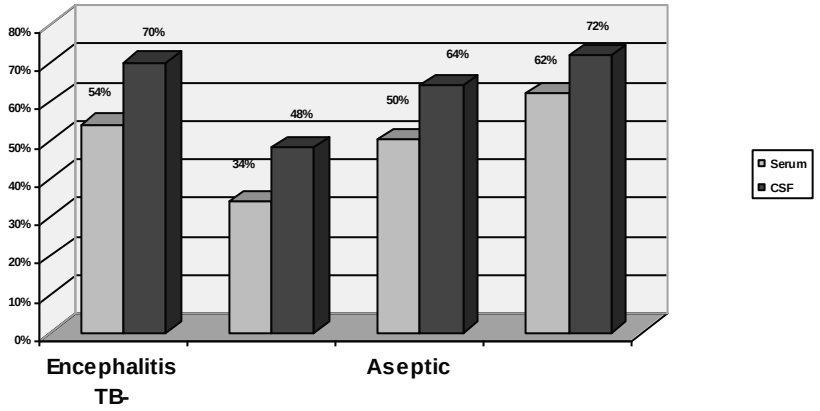

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Figure 2: Detection rates of pneumococcal antigen in sera and CSF in different manifestations of meningitis patients using ELISA.

DISCUSSION

Bacterial meningitis is infection of the subarachnoid space and meninges which is caused by direct spread of bacteria after sinusitis, open fractures and surgery on or near the (CNS) (Laban and Neill 2007) with mortality and morbidity 50% of these cases (Siga´uque *et al*. 2008). *S.*

pneumoniae are responsible for 80% of cases (Beek *et al*. 2006). Having an early diagnosis and starting immediate empirical therapy are the key factors to decrease the morbidity and mortality related to pneumococcal meningitis.

Firstly, in the present study, the presence of *S. pneumoniae* antigen was investigated in serum and CSF samples of meningitis patients using SDS-PAGE under reducing conditions and western technique based on the specific anti *S.* pneumoniae antibody. Our results showed that the protein bands were identified in both serum and CSF samples with very wide range of molecular weight from 18 to 215 kDa.

Several authors separated proteins of *S. pneumoniae* antigen using SDS-PAGE under reducing conditions The most recent García-Suárez *et al*. (2009) who reported that SDS-PAGE and western blot assay with a specific polyclonal antibody was developed for direct detection of the *S. pneumoniae* protein in body fluid samples from children with pneumococcal infections by the electrophoretic profile of their major cell surface proteins. This may be a marker to be used in the clinical diagnosis of pneumococcal infection.

Western blot technique also was used to determine which band corresponds to the reactive epitope that is specific for *S. pneumoniae*. We used a specific anti *S. pneumoniae* antibody to react with the *S. pneumoniae* antigen and the reaction gave an intense sharp band in both CSF and serum samples of infected meningitis patients but no bands were recorded in non infected samples. The molecular weight of *S. pneumoniae* antigen was determined and found to be at 40 kDa. Several authors did not agree with our result in that the *S. pneumoniae* antigen has a low molecular weight. For example Janice *et al*. (1999) used SDS-polyacrylamide gel electrophoresis and western blot technique and reports that *S. pneumoniae* main surface protein with a high molecular weight of 53 kDa. Furthermore, Kanungo *et al*. (2002) used a western blotting and detected a 53-kDa pneumolysin (PLY) which is a protein found in all pneumococcal strains and is useful in pneumococcal antigen diagnosis and suggested that the western blotting is specific method for PLY detection.

Other authors were agree with our results in that the *S. pneumoniae* antigen has a low molecular weight (Morrison *et al* . (2000) and Anthony *et al***.** **(**2003**)**).They showed that the pneumococcal surface adhesin A (PsaA) antigen is a 37 kDa expressed on the surface of *S. pneumoniae* among all *S. pneumoniae* serotypes and is essential for *S. pneumoniae* serotypes virulence. Also, Tharpe *et al*. (1998) identified a pneumococcal surface adhesion protein as a common cell wall protein of *S. pneumoniae* (PsaA) and the identified protein had an apparent molecular weight of 37 kDa as determined by SDS-polyacrylamide gel electrophoresis. Moreover, Tharpe and Russell (1996) and Jennifer *et al* . (1998) reported that *S. pneumoniae* purified protein of an apparent molecular weight of 37-kDa as determined by SDS-PAGE and by western blot. Similar results of a main band of 40 kDa was found in all isolates of *S. pneumoniae* examined using SDS-PAGE and western blot in serum samples detected by Jan and Knut (1996). Mouneimne *et al*. (1997) found that a band at 36 to 38 kDa was recognized by all reactive sera of *S. pneumoniae*. This band was conserved among the different *S.*

pneumoniae serotypes. Beside, Helen *et al*. (2005) reported that a western blot of total proteins from samples infected with pneumococci, probed with anti pneumococci, antibodies showing the appearance of the band of approximately 34kDa. while 54kDa, 39kDa, and 17kDa bands were isolated among the *S. pneumoniae* serotypes in serum samples by Myhrinder *et al*. (2008).

For the diagnosis of *S. pneumoniae* infections blood bacterial culture proved to be a low-sensitive test that depends on viable microorganisms and on previous administration of antimicrobial agents. However, polymerase chain reaction (PCR) is complex and time-consuming, and is very expensive for use in developing countries. Then, immunological assays such as counterimmunoelectrophoresis (CIE), latex agglutination (LA) and ELISA have been proposed as sensitive and specific methods in the diagnosis of *S. pneumpniae* antigens, but, CIE is very expensive for developing countries due to the elevated cost of concentrated pneumococcal omniserum, and LA is also difficult to access due to elevated cost of commercial kits (Henry and Requejo 2007).

Our proposed ELISA proved to be a practical procedure for bacterial antigen detection. And thus, we use ELISA method for detection of *S. pneumoniae* antigen in sera and CSF samples of meningitis patients. We determined the detection rate of The *S. pneumoniae* antigen in both serum and CSF of meningitis patients which were (52%) in sera and (66%) in CSF. These results, which depend on a specific anti- *S. pneumoniae* antibody, showed that the percentage of positivity was high in both CSF and serum samples.

Anthony *et al*. (2005) reported that a high sensitive and a specific method used for diagnosis of *S. pneumonia* in serum samples in adults was achieved with using Enzyme-Linked Immunosorbent Assays (ELISAs). Henry and Requejo (2007) also reported that ELISA was developed for this purpose which was more sensitive than both counter immunoelectrophoresis and latex agglutination for the detection of *S. pneumoniae* antigen in serum, urine, and cerebrospinal fluid. In addition, no false positive ELISA reactions were noted. The increased sensitivity of ELISA was especially valuable in the detection of *S. pneumoniae* antigen following antibiotic therapy, in which case cultures are often sterile and counter immunoelectrophoresis and latex agglutination can be negative. ELISA is thus a valuable tool for the early diagnosis of *S. pneumoniae* infections. Similar observation were obtained by Garcıa-Suárez *et al*. (2007) and Rajalakshmi *et al*. (2008) who reported that pneumococcal antigens in particular, pneumolysin have been investigated as potential diagnostic targets. Pneumolysin antigen detection has been applied to urine (Cima-Cabal *et al*. (2003) and Rajalakshmi *et al*. (2008), serum, and CSF specimens (Kanungo *et al*. (2004). The results have been promising in all, and the detected antigen using ELISA test result in a better diagnostic yield, because of the higher specificity of the pneumolysin detection by using ELISA Garcıa-Suárez *et al*. (2007)and Rajalakshmi *et al* . (2008).

Other authors agreed with our result in that the ELISA was successfully used for diagnosis of serum samples of adults and children

infected with *Streptococcus pneumoniae*. García *et al*. (2003) who reported that the diagnosed samples were 56.6%, and 62.5% in adults and children and this ELISA can provide *S. pneumoniae* antigen tests in the diagnosis of pneumococcal diseases. Beside, Henry and Requejo (2007) agreed with our results when samples of serum from children were assayed by Dot- ELISA for antigen detection. Anti-*S. pneumoniae* omniserum used against 90 serotypes. The detection rate of positive samples of *S. pneumoniae* obtained from serum with ELISA was 58.6% which is similar with our results. Moreover, Scott *et al*. (2002) suggested that Pneumococcal surface adhesin A (PsaA) is a genetically conserved, surface-expressed protein common to all serotypes of *S. pneumoniae* was detected using enzyme linked Immunosorbent assay for diagnosis of pneumococci in infected serum samples with a highly sensitivity which makes this an ideal assay for studies of pneumococcal infections.

On the other hand, Henry *et al*. (2001) suggested that a diagnosis of bacterial meningitis requires isolation of the pathogen from cerebrospinal fluid (CSF) in the majority of patients, the etiology hence is successfully determined. CSF samples from pediatric patients with clinical diagnosis of pneumococcal meningitis were evaluated by Dot-ELISA. This method was standardized in order to detect pneumococcal polysaccharide antigen in CSF samples. Anti-Pneumococcal antibody diluted 1: 200 and was employed for pneumococcal antigen detection. ELISA showed relative indices of 100 percent for sensitivity which could not be obtained from the other body fluids. Furthermore, Keith *et al*. (2008) found that ELISA have high sensitivity in detection of PsaA from convalescent and acute phase in CSF samples which had 85% (Tharpe *et al*. 1998) but measurements of PsaA in acute-phase serum specimens lack sensitivity (Scott *et al*. 2002). In contrast**,** María *et al*. (2007) found that (ELISA) was developed for the detection of pneumolysin in serum and the detected pneumolysin was evaluated using serum from patients with culture confirmed pneumococcal infections in adults (57%) and children (68.7%). In agreeing with our study, Sally *et al*. (1979) used ELISA for the detection of *S. pneumoniae* polysaccharide antigen in cerebrospinal fluid and serum. The ELISA resulted in an increased positivity of the cerebrospinal fluid and serum. ELISA proved to be very useful in the diagnosis of pneumococcal infections; and the antigen was detected in 80% CSF, while 55% had antigen detected in serum by ELISA, 16 h after infection. Serum antigen detection by ELISA correlated with CSF and the detection of *S. pneumoniae* polysaccharide antigens in serum and body fluids has contributed greatly to the rapid accurate diagnosis of pneumococcal infections (Sally *et al*. 1979). Mata and Bernárdez (1996) reported that by ELISA with patients with definite *S. pneumoniae* in serum samples was diagnosed 70.83% in sensitivity and these results suggested that the ELISA is a useful method in rapid diagnosis of *Streptococcus pneumoniae* in infected serum samples. Furthermore, in the present study we found that there is a significant correlation ($r = 0.263$; $p= 0.001$) in the levels of *S. pneumoniae* antigen between serum and CSF of total meningitis patients. This correlation enables us to use serum sample instead of the painful withdrawing of the CSF samples from meningitis patients.

In conclusion, we have identified the 40 kDa protein from *S. pneumoniae* in CSF and Serum with no significant differences between the antigen in serum and CSF and we used Enzyme Linked Immunosorbent Assay (ELISA) in the detection of soluble pneumococcal antigen in serum and CSF samples. So; it is possible to use serum samples in the detection of the *S. pneumoniae* antigen instead of CSF samples.

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تعـيين كيميائي منـاعي لاحد انتيجينات الاستربتوكوكس بنيومونيـا (40 كيلـو دالتون) فـي سيروم و سائل النخاع الشوكي لمصابين باستربتوكوكس بنيومونيا

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**** مركز بحوث التكنولوجيا الحيويه بدمياط الجديده-مصر**

استهدف هذا البحث تعيين كيميـائي منـاعي لاحد انتيجينـات الاستربتوكوكس بنيومونيـا فـي السيرم و سـائل النخـاع الشـوكي ولقد تطرقنـا فـي هـذه الدراسـه الـي التعـرف علـي احـد انتيجينـات بكتيريـا الاسـتربتوكوكس بنيومونيـا فـي عينـات السـيرم و سـائل النخـاع الشـوكي لاشـخاص مصـلبين بالالتهـاب السـحائي باسـتخدام طريقـه الادمصـاص المنـاعي الانزيمـي معتمدين في ذلك علي حساسيه الجسم المضاد للانتيجين الخـاص بـه .وقد لـوحظ عدم وجود اختلاف واضح بين السيروم والسائل النخاعي في نتائج تعيين الانتيجين حيث كان 66% في سائل النخـاع الشوكي و52% في السيروم, مما يجعلاستخدام السيروم ممكن بـدلا من سـائل النخاع الشوكي فـي تعيين الانتيجين .كمـا انـه تم فصـل البـروتين الخـاص بالاستربتوكوكاس بنيومونيا من السيروم وسائل النخاع الشوكي بطريقة البولي اكريلميد جل الكتروفوريزس ثم التعرف علي هذا البروتين بصبغة الكوماسي الزرقاء ووجد وزنـه الجزيئـي 40 كيلـو دالتـون في كل من السيروم وسائل النخاع الشوكي.

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