Evaluation of some immunological markers in children with bacterial meningitis caused by *Streptococcus pneumoniae*

Alaa Younus[1], Saife Al-Ahmer[2] and Majid Jabir[3*]
1. Department of Biomedical Engineering, University of Technology, Baghdad, IRAQ
2. Institute of Genetic Engineering and Biotechnology for Post Graduate Studies, University of Baghdad, Baghdad, IRAQ
3. Department of Applied Science, University of Technology, Baghdad, IRAQ
*msj_iraq@yahoo.com

Abstract
The current study is an attempt to investigate the level of IgG, IgM, IgA, C3 and C4 in patients infected with meningitis. Single radial immune diffusion test has been done to determine the concentration of IgG, IgM, IgA, C3 and C4 in the blood samples of 10 children infected with pediatric bacterial meningitis who had a positive bacterial culture in the CSF with Streptococcus pneumoniae and 15 blood samples from healthy children as a control. The results had shown that there was an increase of the concentration of IgG, IgM, IgA, C3 and C4 in most of the patients in comparison with the control group, which refers to the importance of IgG, IgM, IgA, C3 and C4 in the immunological response against the bacteria responsible of causing this disease.

Keywords: *Streptococcus pneumonia*, Igs, C3, C4.

Introduction

Meningitis remains one of the most feared infectious diseases worldwide and is associated with substantial mortality and long-term neurological complications. It is an inflammation of the fluid and membranes covering the brain and spinal cord[8]. More than 1.2 million people are diagnosed with meningitis annually and this disease is one of the 10 most frequent causes of death worldwide. Therefore, timely diagnosis and treatment of this disease are of particular importance[1,12].

Meningitis may be caused by bacterial, viral and fungal infections, malignancy, medications, e.g. immunosuppressants and chronic inflammatory diseases, e.g. sarcoidosis[9]. Meningeal inflammation can lead to severe neurological complications including altered mental state, seizures, stroke, hydrocephalus, cranial nerve palsies and cerebral herniation[15]. Bacterial meningitis, an inflammation of the meninges affecting the pia, arachnoid and subarachnoid space that happens in response to bacteria and bacterial products, continues to be an important cause of mortality and morbidity in neonates and children[2,13].

However, mortality and morbidity vary by age and geographical location of the patient and the causative organism. Patients at risk for high mortality and morbidity include newborns, those living in low-income countries and those infected with Gram-negative bacilli and *Streptococcus pneumoniae*[3]. Almost all microbes that are pathogenic to human beings have the potential to cause meningitis, but a relatively small number of pathogens including group B *streptococcus, E. coli, Listeriamonocytogenes*, *Haemophilusinfluenzae* type b (Hib), *S pneumoniae* and *Neisseria meningitidis* accounts for most cases of acute bacterial meningitis in neonates and children[5,6].

The types of bacteria causing bacterial meningitis are varying by age group in premature babies and newborns up to three months old. Common causes are group B *Streptococci* (subtypes III which normally inhabit the vagina and are mainly a cause during the first week of life) and those that normally inhabit the digestive tract such as *Escherichia coli* (carrying K1 antigen). Older children are more commonly affected by *Neisseria meningitidis* (meningococcus), *Streptococcus pneumoniae* (serotypes 6, 9, 14, 18 and 23) and those under five *Haemophilus influenzae* type B[10,14].

Severity of illness on presentation, infection with antimicrobial resistant organisms and incomplete knowledge of the pathogenesis of meningitis are additional factors contributing to mortality and morbidity associated with bacterial meningitis[4]. The mortality of untreated bacterial meningitis approaches 100% and, even with optimum treatment, mortality and morbidity might happen. Neurological sequelae are relatively common in survivors of meningitis, particularly after pneumococcal meningitis[13]. The presence of microorganisms in normally sterile body fluid specimens may be representative of life-threatening infections. Infection of normally sterile body fluids often results in severe morbidity and mortality; therefore, rapid and accurate microbiological assessment of these specimens are important to successful patient management[11].

Due to the serious, time-sensitive nature and high mortality of bacterial meningitis, diagnostic tests with a high sensitivity and fast turnaround time are needed. Therefore, this study aimed to investigate the value of IgG, IgM, IgA, C3 and C4 in the serums of the pediatric meningitis patients caused by *S. pneumonia.*

Material and Methods

Collection of Samples: During the period of study from the beginning of November 2017 to the end of February 2018, ten blood samples from children with different ages and

gender confirmed to have pediatric bacterial meningitis caused by *S. pneumonia* have been collected from three hospitals of Baghdad and 15 blood samples from healthy children as a control. All the serums have been tested to determine the levels of IgG, IgM, IgA, C3 and C4 using radial immuno-diffusion method.

Measurement of immunoglobulins, C3 and C4 level: The plate of agarose gel containing the goat antiserum of Igs and C3 or C4 has been removed from its envelope and let to stand at room temperature for few minutes to get rid of any condensed water in the wells by evaporation. Each well of the plate has been filled with 5 µl of a sample and waited to be completely absorbed before handling the plate. The plate has been closed and placed in a moist chamber for 72 hours. The precipitating ring has been measured with an appropriate ruler. The concentration of Igs, C3 and C4 has been obtained by reading the enclosed reference table of the kit corresponding with the precipitating ring diameter.

Statistical analysis: Comparison between groups at one time point was made using unpaired t test (prism Graph 7.04). A P value <0.05 was considered a significant[7].

Results and Discussion

This study included determination of Igs concentration in 10 serum samples of the patients confirmed to have pediatric bacterial meningitis which included the infection with *S. pneumonia.* Also 15 serum samples of healthy children have been tested as control group.

The results of the IgG concentration are illustrated in the figure 1. The results have shown the IgG concentration in the serums as mean ± SEM. In healthy control it was 821.7 ± 13.02 pg/ml. The IgG concentration in the serum of 10 patients infected with *S. pneumoniae* was detected and there was a significant difference (P < 0.05), P value = 0.0004 and the mean ± SEM of the patients was 3122 ± 210.8 pg/ml.

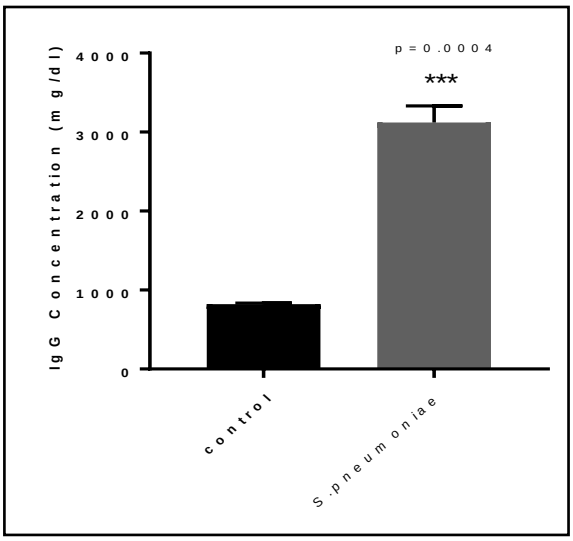

Fig. 1: IgG level in the serum of the patients infected with *S. pneumonia*.

We also measured the IgA concentration as illustrated in the figure 2. Our results demonstrated that the IgA concentration in the serums of the healthy control was 39.67 ± 8.212 pg/ml. while IgA concentration in the serum of 10 patients infected with *S. pneumoniae* was 143.7 ± 5.634 pg/ml and there was a significant difference (P < 0.05), *P value* = 0.0005. IgM level was 134.4 ± 28.82 pg/ml while in healthy control it was 24.33 ± 2.848 pg/ml. As shown in figure 3, determination of C3 and C4 concentration was done using single radial diffusion assay.

The C3 concentration in the serum of 7 patients infected with *S. epidermidis* was 198 ± 33.54 pg/ml while in control group it was 72.67 ± 4.055 pg/ml as in figure 4. The results of C4 level showed a significant increase in C4 level 47.03 ± 2.621 pg/ml while in control group it was 21.67 ± 0.8819 pg/ml as shown in figure 5.

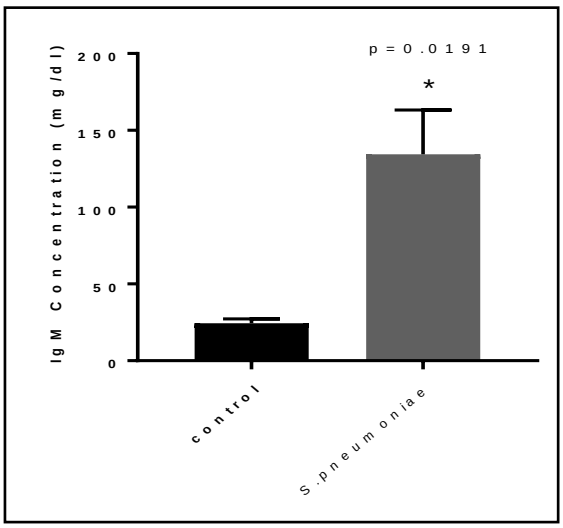

Fig. 2: IgM level in the serum of the patients infected with *S. pneumonia*

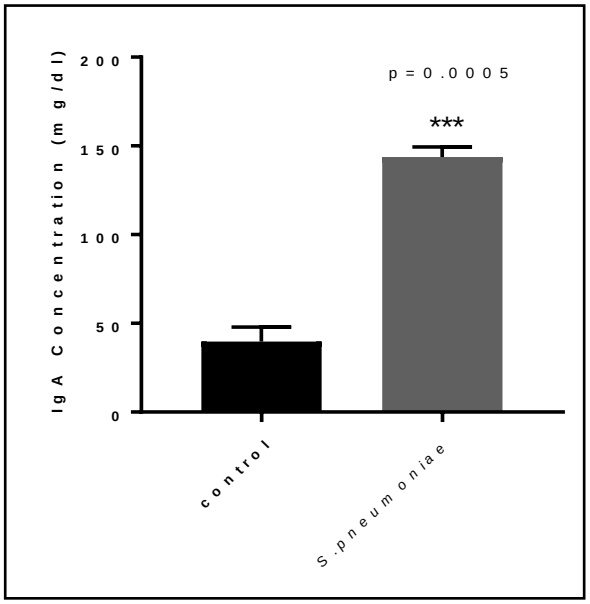

Fig. 3: IgA level in the serum of the patients infected with *S. pneumonia*

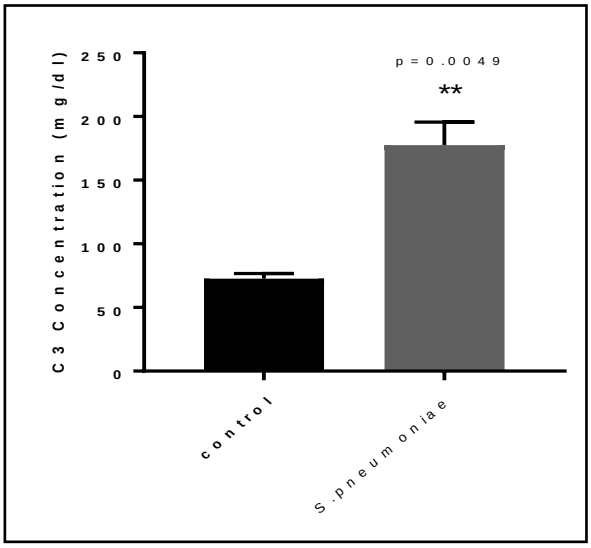

Fig. 4: C3 level in the serum of the patients infected with *S. pneumonia*

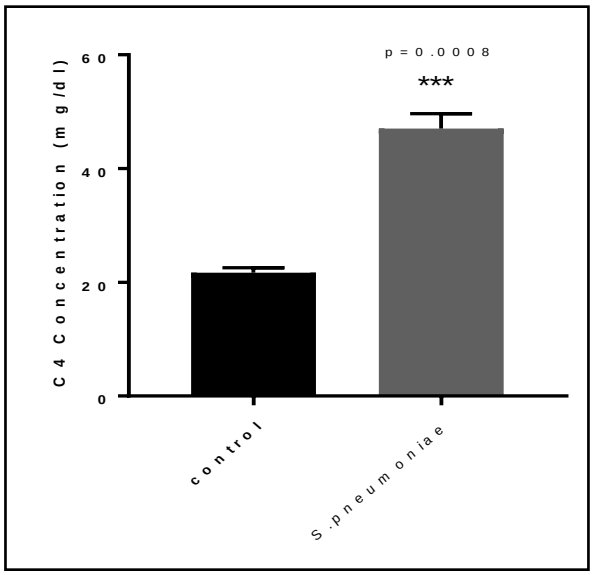

Fig. 5: C4 level in the serum of the patients infected with *S. pneumonia*

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