Libyan Journal of Science & Technology 8:1 (2018) 40–44

Faculty of Science - University of Benghazi

Libyan Journal of Science & Technology

journal home page: www.sc.uob.edu.ly/pages/page/77

Analysis of the relationship between human complement components C3 & C4 deficiency and C– reactive protein levels as an indicator of the increase of microbial infections

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Highlights

- **The complement deficiency in C3 is higher than C4, and the deficiency in females is higher than males.**
- **There is a strong association between complement proteins C3 and C4 deficiencies and elevation of CRP.**

ARTICLE INFO

Article history:
Received 04 July 2018
Revised 16 December 2018
Accepted 22 December 2018
Available online 26 December 2018

Keywords:
Complement deficiencies, C-Reactive Protein, microbial infection, Complements C3 and C4

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ABSTRACT

The complement system is an important component of the immune system that consists of a group of plasma proteins produced by the liver and serving a defense function. As a result, the deficiency in any of the complement system components leads to the loss of the function of the deficient protein causing a specific clinical problem or disease. For that reason, this study was designed to investigate C3 and C4 deficiencies in young patients and to inspect the possibility of using C-Reactive Protein (CRP) test and the variation of C3 and C4 concentrations in children`s blood as an indicator of the onset of infection. This study was conducted in the children hospital in Benghazi city, Libya. The blood samples were collected from 446 children in all hospital units to examine the proportion of complement deficiency among those children and C3, C4 deficiency was set as an indicator of infection occurrence. The results of this study have shown a deficiency in complement proteins C3 and C4 were in 44 (10%) samples and 2 (0.4%) samples respectively, the female candidates had a higher rate of C3 and C4 deficiency than males. In addition, the CRP levels were elevated in 167 (55.9%) cases. Furthermore, the association between the increase of CRP levels and low levels of C3 and C4 was observed in 26 (8.7%) cases and 2 (0.6%) cases. These results suggest a strong association between complement proteins C3 and C4 deficiency and an elevation of CRP levels (P-Value=0.00), which supports the concept of using such association as an indicator of microbial infection.

1. Introduction

The innate immune response is the first line of defense against invading microbes and foreign molecules. Therefore, when a pathogen crosses the anatomical barriers of the host body to internal tissues and blood flow, the innate immune system is activated. The initial host defenses activated in response to the pathogen are host protein pattern recognition receptors such as toll-like receptors (TLRs) which found in the extracellular fluid that recognise and attach to the pathogens through pathogen-associated molecular patterns (PAMPs) (Lee *et al.*, 2013). The antimicrobial enzymes can be activated and act by several mechanisms including lysozymes that digest bacterial cell wall, antimicrobial peptides lysis bacterial cell membrane (Underwood and Bakaletz, 2001) and the proteins of the complement system also mediate microbial cell death by osmotic flux (Xiong *et al.*, 2003). Once the pathogen breached the innate immunity barriers, the host immune system starts to adjust to specifically encounter the invading pathogen through the adaptive immunity. Thus, during the adaptive immune response, phagocytic cells for example, macrophages, dendritic cells and monocytes will direct to the site of infection by a panel of cytokines such as IL-1 (Dinarello, 2011). Phagocytic cells sequentially present the peptides of the degrade pathogen loaded on MHC-II to T helper cells which help to activate other cells of the innate and adaptive immunity (Xu and Banchereau, 2014). Activated B cells (plasma cells) produce antibodies that travel to the site of infection in order to block the binding of microbial antigen to the host cells and facilitate it`s destruction by other arms of the immune system for instance, complement system proteins (Mac Pherson and Austyn, 2012; Sorman *et al.*, 2014).

There are different types of proteins secreted by liver, they are always present in the serum in a constant concentration, and in an inactive form; these proteins are collectively known as the complement system, they so named because they can “complement” or act in conjunction with the adaptive immune defenses (Sorman *et al.*, 2014). The complement proteins become activated in response to certain stimuli triggering a chain of events that enhance the host defenses and promote inflammation resulting in the destruction of invading microbes. The different complement pathways are triggered by different types of molecules such as immunoglobulins (Igs), lipopolysaccharides (LPS), carbohydrates and viral envelopes (Mac Pherson and Austyn, 2012). In more details. The complement system contributes to the overall inflammatory response of the immune system through the production of potent proinflammatory molecules such as C2a and C5a, as well as in the opsonization of target cells via C3b and lysis of the target cell by the terminal product which is the membrane attack complex (MAC) (Dunkelberger and Song, 2010; Sarma and Word, 2012). Most importantly, C3 protein occupies a central position in the complement cascade because it sub-serves several critical functions, the presence of even small amounts of C3 component reduce the severity and frequency of the microbial infections. However, decreased levels of C3

to levels below normal concentration has been linked to autoimmune disorders and/or infections (Ballanti *et al*, 2013). Ghannam *et al*., (2014) have reported that C3 deficiency has been correlated with the history of recurrent pyogenic infections. More to the point is that it has been reported that invasive infections including meningitis, bacteremia, pneumonia, and otitis media with *Streptococcus pneumoniae, Neisseria meningitidis* and *Haemophilus influenzae* were accompanied by a decrease in the complement of C3 concentrations (Ram *et al*., 2010). Deficiency of complement factors (factor I, factor H, CR1, CD59) exposes affected individuals to severe pyogenic bacterial infections; while factor H and factor I deficiencies cause secondary C3 deficiency with C3 consumption and impose the same infections risk as a primary C3 deficiency (Agarwal *et al*., 2014). Microbial infections were reported in individuals with deficiency of the classical pathway protein C4 including *S. pneumoniae, H. influenzae* and *N. meningitidis*. Indeed, classical pathway components together with immunoglobulin G (IgG) and M (IgM) form an efficient weapon against these pathogens. Immunological screening showed that the limited C4 deficiency of C4a and/or C4b, are inherited immune deficiency and have a higher incidence of autoimmune diseases in humans (Ram *et al*., 2010). The complete deficiency of either C4a or C4b is quite common in about 6% of the population (Blanchong *et al*., 2001).

C-reactive protein (CRP) is a conserved plasma protein produced in many sites in the body like the liver (Chandrashekara, 2014) and is normally found at concentrations of less than 1 mg/dL in the blood and this protein serves as an early marker of inflammation or infection (McCabe and Rimington, 1984; Kolb-Bachofen, 1991). High concentration of (CRP) and white blood cells (WBCs) is widely applied as a parameter to support the diagnosis of infection (Sarsu and Sarac, 2016). The CRP concentrations will rise rapidly and reach to peak at levels of up to 350 – 400 mg/dL after 48 hours during the first 6 to 8 hours of trauma, inflammatory diseases and microbial infections (Chew, 2012). CRP recognizes and binds to a specific molecular configuration which is released during cell death or expressed on the surfaces of pathogens and target cells (Sproston and Ashorth, 2018). So, the concentration of CRP increased after hours from tissue injury or infection suggests by rapidly synthesis from hepatocytes (Black *et al*., 2004). CRP plays a vital role in initiating the elimination process of pathogens by interacting with humoral and cellular effector systems in the blood (Ansar and Ghosh, 2013). This study was conducted to investigate the feasibility of using the increase of CRP levels and change the concentrations of C3 and C4 in children`s serum for the detection of microbial infection.

2. Materials and methods

2.1 Samples preparations

The study area focused on patients from a pediatric hospital in Benghazi city, Libya. Children were chosen randomly from all of the units of the hospital and also from the outpatient department (OPD) for both genders. The patients who tested for measurement of CRP levels and for C3 and C4 deficiencies were selected based on a medical history and by referral from a medical consultation to the serology department. Determination of the CRP and the complement components levels were carried out according to their concentrations in the serum of the blood. All blood samples were drawn, preserved and analyzed in accordance with a standard medical technics. In order to obtain blood serum, all whole blood samples were collected in plane tubes, then allowed to clot by leaving them undisturbed at room temperature for 15-30 minutes, tubes were then centrifuged (5000 rpm for 5 minutes). Immediately, serum components were transferred into clean Eppendorf tubes using sterile tips, serum samples were maintained at 2–8 °C prior analyzing, if serum is not analyzed immediately serum should be stored at –20 °C.

2.2 Determination of complement components C3 and C4 levels.

The complement C3 and C4 assay are an immune turbidimetric procedure that measures increasing sample turbidity caused by the formation of insoluble immune complexes when antibody C3 and C4 component are added to the sample. Measuring the C3 and C4 complements were carried out using Roche Cobas Integra 400 Plus systems. The serum samples were incubated with a buffer and a sample blank determination is performed prior to the addition of C3 and C4 antibodies. In the presence of an appropriate antibody in excess, the C3 and C4 concentration are measured as a function of turbidity. The concentration of the complements was interpreted according to the normal levels of C3 (0.9–1.8 g/L) and for C4 (0.1–0.4 g/L).

2.3 Quantitative analysis of CRP in the blood samples.

The measurement of CRP concentrations was carried out by Latex-Agglutination-Test for the determination of qualitative of CRP in serum or plasma. When polystyrene latex particles coated with goat antibody IgG to anti-human CRP in glycine buffer are mixed with a few drops of patient's serum containing CRP higher than the normal concentration (approximately 1.0 mg/dL or greater) in the blood, a visible agglutination (positive reaction) will take place within 2 minutes. The lack of agglutination (negative reaction) indicates the level of CRP is within the normal range. Briefly, using the six places oval ring slide provided, one drop (~35 µl) of the positive control on the first ring and one drop of the negative control in the second ring were placed, the remaining rings were used for patient samples. One drop of the undiluted patient sample and one drop of a 1:5 dilution of the same sample were placed in the next oval rings. Gently the CRP latex reagent was re-suspended and added as one drop to each position containing a sample to be tested. The samples were then mixed with stirring rod and distributed evenly over the entire oval ring. After that, the slides were rotated and rocked in the same motion for three minutes and the results were obtained immediately under direct light.

3. Results

3.1 The distribution of complements C3 andC4 deficiency at the hospital department.

In order to investigate the distribution of complements C3 and C4 deficiency at hospital departments, 446 samples were collected from different hospital words and sections including inpatients wards A, B, and C; Isolation department (ISO); Intensive Care Unit (ICU); Nephrology department (NEPH); Neonatal department (NN); Gastroenterology department (GAST); Surgery department (SURG) and Outpatient department (OPD). The serum samples that showed C3 deficiency at hospital departments are 44 (10%) of total patients. The samples that showed low levels of C3 in females were higher than males 29 (66.2%) and 15 (33.8%) respectively at all departments. The distribution of complement C4 deficiency appear in just two departments of ISO and ICU, they were counted as one patient in each department (0.4%), those two patients were also having a deficiency in both complements C3 and C4. The distribution of the patients in the hospital units did not show significant correlation between C3 and C4 deficiencies (P-value = 0.02) (r = 0.3), (P-value = 0.01) (r = 0.4), Table 1.

Table 1

The distribution of complements C3 andC4 deficiency at the hospital department.

Complement	Low C3				Low C4			
Gender	Male		Female		Male		Female	
Hospital Units	NO	%	NO	%	NO	%	NO	%
Ward (A,B,C)	6	13.6	10	22.7	0	0	0	0
ISO	4	9	4	9	0	0	1	50
NEPH	0	0	1	2.2	0	0	0	0
ICU	4	9	7	16	1	50	0	0
NN	0	0	1	2.2	0	0	0	0
OPD	1	2.2	3	6.8	0	0	0	0
GAST	0	0	1	2.2	0	0	0	0
SURG	0	0	2	5	0	0	0	0
Total	15	33.8	29	66.2	1	50	1	50

3.2 Measurement of CRP in patient`s serum at the hospital departments according to the gender.

In order to investigate the microbial presence and pathogenesis in the body, different routinely microbial diagnosis relies on the increase of inflammations that can be detected by measuring CRP in the blood. Therefore, measurement for CRP concentration was carried out in the different hospital departments. 299 random serum samples were used as a second portion of the serum that used to measure the complements C3 and C4 complement levels. The tested samples were collected from different hospital words and sections including inpatients words A, B and C, ISO ICU, NEPH, NN, GAST, SURG and OPD. The results of CRP measurements have revealed that 167 (55.9%) patients showed elevation in CRP concentrations. Whereas levels of normal CRP were recorded in 132 (44.1%) patients. The distribution of CRP concentrations was variable in all hospital departments. The CRP elevation in females are in all departments, CRP elevation in males has not appeared in GAST, department, Table 2.

Table 2

Measurement of CRP at the hospital departments according to the gender.

CRP	(+ve) CRP				(-ve) CRP			
Gender	Male		Female		Male		Female	
Hospital Units	NO.	%	NO.	%	NO.	%	NO.	%
Units (A,B,C)	41	24.5	38	23	38	28.8	27	20.4
ISO	13	7.8	13	7.7	7	5.3	3	2.2
NEPH	4	2.4	0	0	3	2.2	5	3.8
ICU	15	9	21	12.5	10	7.5	11	8.3
NN	4	2.4	1	0.6	2	1.5	7	5.3
OPD	10	6	5	2.9	6	4.5	11	8.3
GAST	0	0	1	0.6	0	0	0	0
SURG	0	0	1	0.6	1	0.7	1	0.7
Total	167				132			

3.3 Analysis of he association between CRP and C3, C4 component levels.

The low levels of the complements C3 and C4 concentrations have been recorded as risk factors for the increase of microbial infections, therefore, 324 blood serum samples were tested to investigate the interaction between the change in the CRP concentrations in the blood and the levels of C3 and C4 complements (low and normal concentrations). The concentrations were represented as (low- Normal- High) and CRP test represented as (positive and negative). The results of the low C3 measurements showed CRP levels were variable, the samples that detected low C3 and (+ve) CRP are 9 (2.8%) in males and 19 (5.9%) in females, whereas samples that have low C3 and (-ve) CRP were 6 (1.8%) in males and 10 (3%) in females, Table 3. The results of the C4 measurements have revealed that most of the serum samples showed normal and high levels of C4 concentrations, only two samples showed low C4 levels with positivity in CRP for both genders (0.3%), Table 4. In contrast, the normal levels of C3 with (+ve) CRP has recorded the highest number of samples in male 62 (19.1%) and female 52 (16%). Whereas the normal levels of C4 with (+ve) CRP have recorded a predominant number of samples in male 63 (19.4%) and female 67 (20.6%), Table 4. The statistical analysis for a correlation between low levels of C3, C4 deficiency and positive (CRP) was significant, conversely relationship (p-value=0.00, r=0.8) (p-value=0.00, r=0.6) respectively for C3 and C4.

Table 3

Association between CRP and C3 component Levels

C3 Levels	LOW (<0.9 g/l)				NORMAL (0.9 - 1.8 g/l)				HIGH (>1.8 g/l)			
Gender	M		F		M		F		M		F	
	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%
(+ve) CRP	9	2.8	19	5.9	62	19.1	52	16	26	8	18	5.5
(-ve) CRP	6	1.8	10	3	46	14.2	51	15.7	16	4.9	9	2.8
Total	15	4.6	29	9	108	33.3	103	31.8	42	13	27	8.3

Table 4.

Association between CRP and C4 component Levels

C4 Levels	LOW (<0.1 g/l)				NORMAL (0.1 – 0.4 g/l)				HIGH (>0.4 g/l)			
Gender	M		F		M		F		M		F	
	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%
(+ve) CRP	1	0.3	1	0.3	63	19.4	67	20.6	33	10	21	6.7
(-ve) CRP	1	0.3	0	0	41	12.6	56	17.3	26	8	14	4.3
Total	2	0.6	1	0.3	104	32.1	123	38	59	18.1	35	10.8

3.4 Distribution of low C3 and C4 *vs* positive CRP at hospital departments

Because of the positive of CRP as an indicator for infections, 299 samples from the different hospital words and have +ve CRP was introduced to investigate the interaction between C3 and C4 deficiencies and positive CRP. The results have reviled that only three hospital words showed elevation of CRP within complement deficiency of C3, the departments that involved in low C3 and +ve CRP are ICU, Units A, B, C, Isolation department 10, 8 and 3 (43.5%, 34.8%, 13%) respectively. Whereas C4 deficiency and +ve CRP appeared just in one patient in the isolation department, Table 5.

Table 5

Distribution of low C3 and C4 *vs* positive CRP at hospital departments

Comparison C3&C4 *Vs* CRP	Low C3 *Vs* (+ve) CRP		Low C4 *Vs* (+ve) CRP	
	NO.	%	NO.	%
Units (A,B,C)	8	34.8	0	0
ISO	3	13	1	100
NEPH	0	0	0	0
ICU	10	43.5	0	0
NN	0	0	0	0
OPD	0	0	0	0
GAST	1	4.3	0	0
SURG	1	4.3	0	0
Total	23	100	1	100

4. Discussion

It has been well documented that several types of infections such as HIV, herpesvirus and pneumonia are amongst the leading causes of the increased mortality and morbidity rates (Finnegan *et al*., 2014). Thus, it has been always a necessity to establish a new reliable laboratory detection methods to identify the presence of infectious agents and consequently accurate diagnoses of infectious diseases. For that reason, a number of routine laboratory tests have been used as indicators of microbial infection; tests such as WBCs count, erythrocyte sedimentation rate (ESR), procalcitonin (PCT), CRP, serum amyloid A (SAA) and plasma viscosity (Michail *et al*., 2013). Clinically, increased levels of CRP can be used as a sign of acute inflammation as a result of an infection or tissue damage (Sproston and Ashworth, 2018); as well as monitoring of autoimmune diseases (Pepys and Hirschfield, 2003). In addition, it has been shown that CRP has an important role in host defense by complement opsonization of antigens and target cells by activating C1 protein, which sequentially activates the cascade of complement pathway (Gershov, *et al.*, 2001; Szalai, 2002). Because of the availability and low cost of CRP test, physicians in the children hospital in Benghazi city use it as a routinely reliable tool to investigate a panel of infectious diseases and as an indicator of inflammation. Other scientists have attempted to evaluate the role of CRP in the diagnosis of sepsis in different patients (Van Gestel *et al*., 2004). The analysis of CRP in Benghazi children hospital demonstrated that the hospital words A, B, and C, Isolation and ICU departments showed the highest rate in CRP levels, and this was an expected because of the patient`s medical conditions. The levels of CRP according to the gender were variable at all hospital departments (>10 mg/L). The increase of serum CRP of the admitted patients in ISO and ICU could be used to predict bacteremia. This because a previous study by Presson and colleagues showed that CRP and inflammations can be used as a sign of bacteremia within 48 hours of fever in neutropenic patients (Presson *et al*., 2005). Many patients were dropped from the investigation because their CRP levels were unchecked, either because the patients had no fever or seemed uninfected when the investigation for infection was performed straightforward. The results of the investigation might, therefore be hampered by selection bias and sampling error and should be carefully interpreted. Moreover, measurement of complement components C3 and C4 have provided an initial image of complement concentration among patients, accordingly, around 11.4% of patients had low concentrations of C3, and this makes them more susceptible to infections. More to the point, many studies have shown that deficiency of C3 component has a correlation with some invasive infections, namely, meningitis, bacteremia and pneumonia; as well as being related with autoimmune disorders (Agrawal *et al*., 2014). In the current study, C3 and C4 deficiency were considered as additional markers for microbial infections and this notion has been supported by earlier studies concerning meningococcal disease (Lisa and Ram, 2014), Aspergillosis (Speth and Rambach, 2012), increased susceptibility to recurrent infections with Neisseria (Hanna *et al*., 2015), increased susceptibility to recurrent respiratory infections and infections caused by encapsulated organisms for instance, *S. pneumoniae, N. meningitidis,* and *H. influenzae.* Additionally, patients who have shown a complement deficiency also showed 11% increase in CRP levels and this supports the increased probability of infectious diseases and inflammation. Subsequently, this study demonstrated a correlation between CRP as an indicator of microbial infection and complement components C3 and C4 deficiencies.

Lastly, to our knowledge, there has been no published work in Libya regarding the correlation between complement components deficiency and CRP as indicators of infections; or at least we were unable to find one neither electronic copy nor in a hard copy. Therefore, more research on the prevalence of C3 and C4 deficiencies and study of microbial isolation may assist physicians to make a clear image on the possibility of increased microbial infection due to complement deficiencies.

Acknowledgments:

We would like to express our thanks to all people from the serology department in children hospital for their assistance. We would like also to thank Professor Amina.S. Abusedra for her support and reviewing this research.

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