Detection of Serum Hs CRP and C3 Complement Levels Following Immunization with Measles Containing Vaccine in Iraqi Young Adults

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Abstract

Background: Measles remains a major cause of worldwide childhood mortality, and has been targeted by the WHO for global eradication following the eradication of poliomyelitis. Despite the implementation of mass school catch-up campaigns for measles, an outbreak of measles occurred mostly affecting the adult population.

Objectives: To measurement of Highly Sensitive C Reactive Protein (Hs CRP) and C3 Complement levels in adult volunteers after vaccination with live attenuated measles containing vaccine, and to compare the results with that following vaccination.

Subjects and methods: A sero-surveys for serum Hs CRP and C3 complement levels was conducted among [190] healthy young adults aged 18-25 years, randomly selected, and to compare after a national campaign with measles containing vaccine. Hs CRP and C3 complement were detected in volunteer's sera prior to, 1, and 4 weeks after vaccination, measured by Enzyme Linked Immunosorbent Assay and Single Radial Immunodiffusion Assay respectively.

Results: There was a significant differences ($p \leq 0.05$) in the concentration of serum Hs CRP and C3 complement at week one following vaccination with measles containing vaccine, and returned to normal after four weeks.

Conclusions: Raising Highly Sensitive C Reactive Protein and Complement C 3 Levels in the sera of adult volunteers after measles containing virus vaccine administration could play a role in increasing innate immune response against measles virus infection and may explain some mechanisms of immune response associated with measles infection.

Key wards: HS CRP, C3 complement, measles vaccination.

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Introduction

Measles is a highly contagious viral infection, its transmission can be prevented through high population immunity (≥95%) achieved by measles vaccination [1]. Although fever is a common manifestation of viral infections, elevated CRP occurs infrequently and has often been used to distinguish bacterial from viral infections [2]. Since the rash of measles may be a manifestation of the cellular immune response to this virus, it seemed likely that CRP might be increased at least transiently during viral infection [3].

The recognized key role of the innate immunity in the induction of cognate immunity led to explore further the possible contribution of complement in MV infection [4, 5]. Sissons and co-workers observed that MV Edmonston strain-infected human HeLa cells activate, in the absence of antibodies, the alternative pathway of human complement and that these cells were more sensitive to complement-mediated lysis [6].

Vaccination with the monovalent or bivalent measles virus vaccine (MVV), or the increasingly used and cost-effective trivalent vaccine has led to a dramatic reduction in the morbidity and mortality associated with measles [7]. In order to evaluate the vaccine efficacy it is important to know the immune response of individuals against measles containing virus vaccine.

Prior to the introduction of vaccine, most people acquired immunity through infection with wild measles virus during childhood [8]. Measles morbidity had been reduced greatly since measles containing vaccine [MCV] was introduced in 1966[7]. A national estimate of measles immunity and an understanding of predictors of measles susceptibility are essential for assuring sustained elimination of endemic disease[9] .

The population immunity did not reach the presumed thresholds of measles elimination. The significant decay of antibody against measles was found since adolescences. Secondary school students can acquire sufficient immunity to measles after revaccination program which can be conducted effectively and practically [10]. Some adults may not have received full protection due to changes in the MMR vaccine [11]. In order to prevent measles in adults, high-risk groups must be identified and catch-up for selected groups considered.

The current study aimed for clinical follow up and identification of immunity reflected by Hs CRP and C3 complement levels in adult individuals, after the administration of the available measles containing virus vaccine.

Subjects and Methods

A cross – sectional study, was conducted in Diyala Medical College. This study was done in Department of Microbiology, in cooperation with laboratory of research Center. And was approved by an institutional review committee and informed consent was obtained from each volunteers prior to enroll in the study. Sero-survay was done for 190 selected random sample of medical students, aged 18-25 years. During National measles, mumps and rubella campaign 2011. 10 subjects were lost during follow up study for variable reasons. The volunteers were subdivided into two groups.

Group 1: included 155 healthy medical students, of them 57 [36.8%] were males, and 98[63.2%] were females were injected with measles containing vaccine (MCV).

Group 2: [control] Included 25 individuals, they were injected with the diluent supplied with the vaccine [placebo]. There mean age group was (20.8) year. 69.2% of them were females and 30.8% were males.

Clinical follow up and oral temperatures were recorded for 14 days after injection.

Both groups were followed up for four weeks after vaccination.

Serum samples were collected and analyzed from the study prior to their submission to vaccination by MCV during National measles, mumps and rubella campaign 2011. Another serum samples were collected 1 and 4 weeks following that campaign.

Estimation of serum Hs CRP and C3 complement:

The volunteers serum had been tested for C-reactive protein by high sensitive Enzyme linked Immunosorbent Assay [Human CRP ELISA kit, BioCheck] and according to manufacturer instructions as follows: After incubation with serum, specific goat anti-CRP antibody are bound to the antigen on the inner walls of the microtiter plate. Excess serum is removed by a washing step, and conjugate (anti-IgG-HRP) is added. After further washing, the substrate (TMB) is pipetted. A blue colour will develop. The action of the enzyme on the substrate is stopped by the addition of 1 M H2SO4, and the blue colour changes into yellow. Absorption is measured by an ELISA reader at 450 nm, then the mean absorbance values was converted to proper concentration in mg/l using a special table provided with each kit.

Radial Immunodiffusion assay was used for quantitative determination of C3 complement using Single radial immunodiffusion test (SRID-endoplate kits). It was done according to manufacturer instruction (Sanofi diagnostic, Pasteur, France) as follows; 5 micro l from volunteers sera were placed in wells then incubated at room temperature for 48 hrs, Immuno-precipitin rings diameters were measured using a reading device. Then the endplate rings of precipitin diameters converted to proper concentration in mg/ml using a special table provided with each kit, when the test was done under standard condition provided to that kit.

Statistical analysis

The results expressed as absolute number, percent, mean ± SD. The data analyzed using Student's "t" test [one paired, two tailed] taking the $p \leq 0.05$ as lowest limit of significance these are completed using Microsoft Excel 2010.

Results

All of the student volunteers were medication free during the entire follow up course. All individuals received the MCV vaccine experienced of local pain and erythema at the site of injection, started within 24 hour and last for 24-48 hour without any residual effect (only one female student suffered from intense pain, itching and erythema last for 72 hour). No one had fever or rash during the follow up period after vaccination, No any other adverse reaction were encountered in our subjects during the entire course of the study. No any clinical or adverse effect appeared in subjects given diluent supplied with the vaccine.

Table 1 shows that Hs CRP Levels as measured by ELISA test in serum of volunteers before, 1, and 4 weeks after vaccination with live measles containing vaccine. The mean serum concentration of group 1 was 2.60 $\pm$ 0.43 mg\l before vaccination, at week 1 an increase in the concentration of Hs CRP was observed (7.35 $\pm$ 1.17) .after 4 weeks the concentration was (2.58 $\pm$ 0.41).

Table (1): Effect of Measles Containing Vaccine on Hs CRP Levels.

Vaccinee	Hs CRP Concentration Mg\l (mean + SD)		
	pre	Post vaccination (Week)	
		1	4
Group I	2.60 ± 0.43	7.35 ± 1.17	2.58 ± 0.41
Group II	2.62 ± 0.44	2.65 ± 0.5	2.63 ± 0.5

Data analysis showed that there was a significant difference of the mean serum CRP concentration among adult vaccines during the first week after vaccination P<0.01 (table 2). No difference in the concentration of CRP was observed in group 2. This is indicating that the association between the two periods (before and after) according to the diagnosis of the studied cases would be very informative.

Table (2): Hs CRP levels between prevaccination and postvaccination among selected individuals.

C.S	Pre		Week 1		P value
S	Mean	STD	Mean	STD	0.001
	2.60	0.43	7.73	1.17	
N.S	Pre		Week 4		0.67
	Mean	STD	Mean	STD	
	2.6	0.43	2.58	0.41	
S	Week 1		Week 4		0.001
	Mean	STD	Mean	STD	
	7.73	1.17	2.58	0.41	

STD: standard deviation, C.S.: comparison significance, N.S.: Not significant
S.: Significant, P value: probability of chance factor to be the origin of difference.

Table 3 shows that the level of serum C3 as measured by SRID test in adult volunteers before, 1, and 4 weeks after vaccination with live MCV. The mean serum C3 complement concentration of group 1 was 126.7±10.2 mg\dl before vaccination, at week 1 and increase in the concentration of C3 was observed (140.5±5.3). after 4 weeks the C3 concentration was (127.1±9.5). Data analysis showed that there was a significant difference of the mean serum C3 concentration among Group 1 P<0.01 (table 2). This is indicating that the association between the two periods [before and after] according to the diagnosis of the studied cases would be informative. No difference in the concentration of C3 was observed in group 2.

Table (3): Effect of measles containing vaccine on C3 Complement Levels.

Vaccinees	C3 Complement Concentration Mg\dl)mean + SD)		
	Pre	Post vaccination (Week)	
		1	4
Group I	126.7 ± 10.2	140.5 ± 5.3	127.1 ± 9.5
Group II	128.6 ±11.5	127.5 ± 9.6	127.4 ± 10.5

Table (4): C3 complement levels between prevaccination and postvaccination among adult individuals.

C.S	pre		Week 1		P value
S	Mean	STD	Mean	STD	0.001
	126.7	10.2	140.1	5.3	
N.S	pre		Week 4		0.8
	Mean	STD	Mean	STD	
	126.7	10.2	127.1	9.5	
S	Week 1		Week 4		0.001
	Mean	STD	Mean	STD	
	140.1	5.3	127.1	9.5	

STD: standard deviation, C.S.: comparison significance, N.S.: Not significant
S.: Significant P, value: probability of chance factor to be the origin of difference.

Discussion

Our country is one of the countries with sustained large-scale immunization program, which offer measles vaccine at 9 month and MCV at 15 month as multivalent vaccine [12].

Following vaccination, the only adverse event was the mild local reaction at the injection site; otherwise MCV was clinically safe, and at the same time effective, and there's enhancement of innate immune response as there is increase in the concentration of CRP and C3 complement. A study conducted previously showed a significant changes in antibody levels was observed after measles vaccination [13] .In this study, there was a significant difference of the mean serum CRP concentration among adult vaccinees during the first week after vaccination. CRP is a member of the class of acute-phase reactants, as its levels rise dramatically during inflammatory processes occurring in the body. This increment is due to a rise in the plasma concentration of IL-6, which is produced predominantly by macrophages as well as adipocytes [14, 15]. CRP binds to phosphocholine on microbes. It is thought to assist in complement binding to foreign and damaged cells and enhances phagocytosis by macrophages [opsonin-mediated phagocytosis], which express a receptor for CRP. It is also believed to play another important role in innate immunity, as an early defense system against infections [14, 15].

There was a significant difference in the concentration of serum C3 complement at week 1 following receipt of live measles containing vaccine and returned to normal level after 4 weeks of vaccination, while no change in the concentration of the group that received placebo during follow up period. This reflect that the presence of C3 component of complement in the serum may in some way increase immune response.

Complement-mediated elimination of lymphocytes and macrophages infected with CD46-down-regulating MCV, may limit the spread of MV infection, and could thus represent an attenuating factor for measles virus. A study of lymphocyte markers, suggested that the complement regulator known either as membrane cofactor protein [MCP] or CD46 is the MV receptor [16]. CD46 is down-regulated in measles virus infected cells, this confers sensitivity to activated complement, regardless of the pathway of activation, and the specificity of the activating antibodies. Interestingly, down regulation of CD46 alone is sufficient to confer susceptibility of infected cells to complement lysis [17].

It has been shown that MV activates the alternative complement pathway with some neutralizing effect. This could play an important role, in vivo, to slow virus propagation by inactivating circulating virus and eliminating MV-infected cells, and to enhance the induction of a specific immune response by opsonizing MV antigens. A critical role of C3 in the pathology induced by gammaherpesviruses has recently been demonstrated [18]. They propose that the activation of the alternative pathway is a primary mechanism of defense against MV infection, before the production of neutralizing anti-MV antibodies by the immune system [19].

The study concluded MCV was clinically safe, and at the same time effective, and there's enhancement of innate immune response as there is increase in the concentration of CRP and C3 complement. In order to prevent measles in adults, high-risk groups must be identified and catch-up for selected groups considered, and appropriate measles control strategies in younger populations seem to be effective in preventing measles in adults.

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