The Levels Of Some Serum Complement Among Seropositive Individuals After Measles Vaccination

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ABSTRACT:

BACKGROUND:

Measles remains a major cause of worldwide childhood mortality. The use of current live attenuated vaccine control the disease, and measles has been targeted by the WHO for global eradication following the eradication of poliomyelitis.

Objective:

To determine the C3 and C4 complement response to measles virus in seropositive volunteers after vaccination with live attenuated measles vaccine.

METHODS:

Thirty seven measles virus seropositive normal volunteers have been enrolled in this study, they were 25 males and 12 females, their age ranged between 15-45 years. 22 of them were vaccinated with measles virus vaccine and 15 were injected with diluent supplied with measles virus vaccine (placebo).

C3,C4 complement against measles virus were detected in volunteer's sera prior to, 1,2,3 and 4 weeks after vaccination, using Radial Immunodiffusion Assay method.

RESULTS:

There was a significant difference in the concentration of serum C3 complement at week 3 , following receipt of live measles virus vaccine in seropositive individuals, while no marked change was observed in the concentration of C4.

CONCLUSION:

Rising C3 level in the sera of seropositive volunteers after measles virus vaccine administration play a role in increasing immune response against measles virus infection.

KEY WORDS: complement, seropositive, measles vaccination

INTRODUCTION:

Measles is one of the diseases that targeted for global eradication by vaccination [1].Most strategies for reducing global measles morbidity and mortality and eliminating measels, are based on the ability to enhance immune response to measles virus (MV) [2]. 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 [3]. In order to evaluate the vaccine efficacy it is important to know the immune responce of individuals against measles virus vaccine. Immunological responce have been studied in seropositive indiveduals, after the adminstration of the available measles virus vaccine, as a preparation for an anticipated measles eradication programme in this country [4].

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The purpose of this study is to determin the level of C3 and C4 complement in the sera of seropositive volenteers after measles vaccination with live attenuated measles vaccine.

MATERIALS AND METHODS:

Subjects:

A total 37 apparently healthy volunteers were included in this study, their ages ranged from 15-45 years,(25 males and 12 females).

The volunteers were age and sex matched, and were subdivided into two groups.

Group 1: Included 22 individuals, they were vaccinated with measles vaccine. The mean age group was (30.2) year. 69.2% of them were males and 30.8% were females.

Group 2: (control) Included 15 individuals, they were injected with the diluent supplied with measles vaccine (placebo). The mean age group was (27.8) year. 69.2% of them were males and 30.8% were females.

Blood sample collection and preparation:

Two- 2.5 ml venous blood was aspirated under

sterile technique from each subject in the studied groups, prior to and 1,2,3 and 4 weeks after injection of vaccine or diluent supplied with the vaccine; and was placed in dry tube for separation. The separated sera were dispensed into closed-capped tubes in 0.2 ml aliquots and stored at –20ºC till tested.- Estimation of serum C3,C4 complement:

Radial Immunodiffusion assay was used for quantitative determination of C3 and C4 using SRID-endoplate kits, to increase the accuracy and precision Mancini or end point technical approach was used, although endoplate RID-kits were designed to be used with either the Mancini (end point) technique or the Fahey & McKelvey (timed diffusion) technique which done as follows:

Procedure of SRID: It was done according to manufacturing instruction; endoplate and references were removed from refrigerator, to equilibrate the reagents to room temperature. Reference sera, controls and subjects samples were mixed in their own containers thoroughly by inversion.

A 5uL of reference sera, control and subjects samples were dispensed each into the appropriate wells, then lid was firmly replaced and incubated at room temperature on a level surface for 48 hours. 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.

RESULTS:

Table 1 showes that the level of serum C3 and C4 as measured by SRID test in seroposetive volenteers befor and 1, 2, 3, and 4 weeks after vaccination with live MVV. The mean serum C3 complement concentration of group 1 was 132.31 befor vaccination, at week 3 an increase in the concentration of C3 was observed (154.79) . Data analysis showed that there was a significant difference of the mean serum C3 concentration among seropositive vaccinees (table 2).

No marked change in the concentration of serum C4 in both groups befor and after vaccination (table 3).

No difference in the concentration of both C3 and C4 was observed in group 2.

DISCUSSION:

By virtue of its numerous advantages, such as simplicity, specificity, sensitivity and economy, SRID has been used for C3 and C4 quantitation in the sera of all the selected individuals.

Serum level of C4 was not markedly changed following administration of measles vaccine. There was a significant difference in the concentration of serum C3 complement at week 3 , following receipt of live MVV , while no change in the concentration of neither C3 nor C4 in the group that received placebo. In my knowledge, there is no reported data concerning the relation of C3 and C4 level in the sera of MV infected persons or even the vaccinated individuals.

It has been found that specific measles antibody titer raised in the serum of mice vaccinated with the DNA expressing a fusion of measles Haemagglutinin protein and complement component C3d [(5).] This reflect that the presence of C3 component of complement in the serum may in some way increase immune response. Moreover, data from other study showed that all the studied subjects have preexisting antibody to measles prior to injection of the vaccine, a significant change in antibody levels was observed during first and fourth week after vaccination [(6).]

A study of lymphocyte markers, suggested that the complement regulator known either as membrane cofactor protein (MCP) or CD46 is the MV receptor [(7).] 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 [(8).]

Complement-mediated elimination of lymphocytes and macrophages infected with CD46-down-regulating MVV, may limit the spread of MV infection, and could thus represent an attenuating factor for measles virus [(8).]

So, the results presented in this study confirm the role of C3 in increase immune response after MVV administration in the study group.

Table (1) Effect of MV on C3, C4 Complement Levels

Vaccine	Complement	S. Complement Concentration Mg / dl (Mean + SD)				
		Prevaccine	Post vaccine (wk)			
			1	2	3	4
Group 1 Live (22)*	C3	132.31±31.26	124.92±31.29	141.43±28.57	154.79±31.03	133.00±25.43
	C4	30.16±5.35	30.3±5.31	31.31±4.52	31.90±4.18	30.79±4.74
Group 2 Placebo (15)*	C3	134.18 ± 23.27	136.78± 23.71	134.45±21.07	135.91±21.35	135.23±21.94
	C4	30.42 ± 4.71	31.05±4.71	31.32±4.97	31.39±4.54	31.20±4.46

* Numbers in parenthesis indicate number of individuals studied

Table (2)c3 levels between prevvaccination and postvaccination among seropositive individuals

C.S	Pre		Week 1		P value
	Mean	Variance	Mean	Variance	
N.S.	132.31	31.26	124.92	31.29	0.44
	Pre		**Week 2**		
N.S.	Mean	Variance	Mean	Variance	0.32
	132.31	31.26	141.43	28.57	
	Pre		**Week 3**		
N.S.	Mean	Variance	Mean	Variance	0.2
	132.31	31.26	154.79	31.03	
	Pre		**Week 4**		
N.S.	Mean	Variance	Mean	Variance	0.93
	132.31	31.26	133.00	25.43	
	W 1		**W 3**		
S.	Mean	Variance	Mean	Variance	0.003
	124.92	31.29	154.79	31.03	

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

Table (3)c4 levels between prevvaccination and postvaccination among seropositive individuals

C.S	Pre		Week 1		P value
N.S.	Mean	Variance	Mean	Variance	0.93
	30.16	5.35	30.3	5.31	
N.S.	Pre		Week 2		0.45
	Mean	Variance	Mean	Variance	
	30.16	5.35	31.31	4.52	
N.S.	Pre		Week 3		0.24
	Mean	Variance	Mean	Variance	
	30.16	5.35	31.90	4.18	
N.S.	Pre		Week 4		0.69
	Mean	Variance	Mean	Variance	
	30.16	5.35	30.79	4.74	

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

REFERENCES:

1- Robertson, S. E., VallMayans, M., Horsfall, S., Wright, P.F., Clemens, J., Ivanoff, B., Lambert, P. H.: The WHO Global programme for vaccines and immunization vaccine trial registry Bull. WHO. 1997; 75,295-303.

2- Moss, W.J.,Polack, F.P. Immune responses to measles and measles vaccine : Challenges for measles control. Virol Immunol. 2001; 14,297-309.

3- Smith, K. Preventing MMR. Pluserix, Smith Kline Biologicals, Belgium 1989 ;9-21.

4- Latif, I.I. Measles vaccination among seropositive individuals: An in vitro and in vivo virological and immunological study. PhD thesis Dept. of Microbiology, College of Medicine, Al-Nahrain University 2002.

5- Green, T.D., Newton, B.R., Rota, P.A., Xu, Y., Robinson, H.L., Ross, T.M. C3d enhancement of netralizing antibodies to measles hemagglutinin. Vaccine. 2001; 20, 242-248.

6- Latif, I.I., Al-Omar, L.S., Abdul-muhymen,N.: Antibody response among seropositive individuals after measles vaccination. Iraqi J Med Sciences. 2005; 3, 26-28.

7- Naniche, D., Varior Krishnan, G., Cervoni, F., Wild, T.F., Rossi, B., Rabourdin Combe, C., Gerlier, D. Human membrane cofactor protein (CD46) acts as a cellular receptor for measles virus. J Virol. 1993; 67, 6025-6032.

8- Schnorr,J.J., Dunster, L.M., Nanan, R., Schaulies, S.J., Schaulies, S.S., TerMeulen, V. Measles vitus indused downregulation of CD46 is associated with enhanced sensitivity to complement mediated lysis of infected cells. J Immunol. 1995; 5,976-984.