A Modified single redial hemolysis diffusion technique for the detections of Cytomegalovirus and Rubella virus IgG and IgM antibodies

AbdulLatif M. Ahmed
Research Centre for Biotechnology, Al-Nahrain University

Abstract

Early diagnosis of rubella and CMV infection is very important mainly in pregnant women, since rubella and CMV cross the placenta causing an infection to the fetus, serous damage may be done to the fetus such as malformation and even fetal death [1,7].

The need for simple test depending on one serum sample (all serological methods for the diagnosis of viral diseases depend on 2 to 3 serum samples), such test, rapid accurate, specific, two coast, easy to performed, quantitative and qualities still need to exists.

In this work, we introduced two modifications to the SRHD making this test able to differentiate IgG and IgM antibodies and such test can adapted to all viruses antibodies (IgG,IgM) to rubella we were detected from antibody titer 1/8 to 1/1024 and CMV were detected from antibody titer 1/8 to 1/512 can be determined in a single reaction using SRHD, which is repaid, accurate and a specific laboratory diagnosis

Introduction

Specimens for serological diagnosis of virus diseases should be collected as early as possible in the disease , and a second specimen collected 10-14 days later, in some cases a third specimen may be necessary after about six weeks as in case of CMV infection. A single specimen is seldom of any value [1]

Rubella virus and CMV antibodies were detected by many methods, such as ELISA, Westerns blot, shell vial, immunofluorescant (IF), polymerase chain reaction (PCR), complement fixation (CF), passive-hemagglutination (PHA) test. [2,3,4,5,6,9];

Human cytomegalovirus (CMV) is an enveloped double-stranced DNA virus belonging to the herpesviridae family. Rubella virus is an enveloped single-stranded RNA virus belonging to the Togaviridae family. CMV and Rubella virus is found drough out the world and human of all ages and sex are susceptible. Mos. CMV and Rubella infections are asymptomatic; however infections can be severe in neonates and immunocompromised individuals. CMV inclusion disease occur in infants dying soon after birth with anemia and hepatosplenonegaly; in older children and adults, associated with chronic diseases such as fibrocystic disease of the pancreas or leukemia in apparent infection is common and maybe detected only by examining post- mortem material. The lesions occur particularly in salivary glands but are frequently found also in lungs, liver, kidneys and pancreas. The consist of characteristic enlarge cells with nuclear and sometime cytoplasmic inclusions. Rubella virus disease which is important because of abnormalities in infants whose mothers have had rubella during early pregnancy. Other viruses cause Illness which may be clinically indistinguishable from rubella and CMV [3,7,8,9] .

Materials and Methods

Specimens

Negative sera (20 samples) and Positive sera tested by CFT and ELISA against rubella (73 samples) and CMV (50 samples) were obtained from central public health laboratory (CPHL) Baghdad.

Antigens

Obtained from local market (Virion) which is commercially available.

Detection of IgG and IgM

All positive and negative sera were divided into two groups, and then one group treated with equal volume of PBS, the second group treated with 0.02 m 2-mercapto ethanol (2ME) (Organon); all mixture were incubated for two hours at room temp .

Complement fixation test (CFT)

A modification of the method describe by Grist et. al [1974] was followed

Passive-single radial hemolgis diffusion (SRHD) a modification of the method describe by Russel and Boyse [1975] .

Tanning and sensitization of sheep RBC (SRBC)

In general the method of Planner *et al*(1977)[12] was followed however, the optimal concentration of tannic acid used in this test was a 1:10000,equal volumes of tannic acid and 2.5 % SRBC, then a final concentration of both rubella Ag. and CMV Ag were 1:16,these suspensions incubated for 30 min. at 37°c ; then a complement was added, to this suspension equal volumes of 2% agarose in PBS, and mixed immediately poured into a petridish, finally a wells of about 3 mm in diameter were cut in that gel to be filled with the treated and non treated sera with 2ME .

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Results and Discussion

The evaluation of the different laboratory methods for the detection of rubella virus and CMV antibodies was determined. Table 1 shows the antibody titer as identified by CFT, ELISA, and SRHD; the antibody titer were detected for 50 CMV positive serum samples at a dilution of sera from 1:8 to 1:64 by CFT; 1:8 to 1:256 by ELISA and 1:8 to 1:512 by SRHD; while the antibody titer for 73 rubella positive serum samples detected as follows 1:8 to 1:128 by CFT; 1:8 to 1:512; and 1:8 to 1:1024 by SRHD.

Table (1) Evaluation of antibody titer of serological methods

Virus No. of samples	Test by	Antibody titer *							
		1:8	1:16	1:16	1:32	1:64	1:128	1:512	1:1024
CMV (50)	CFT		+	+	+	-	-		-
	ELISA		+	+	+	—	+	-	-
	SRHD	—	+	+	+		+	+	-
Rubella (73)	CFT	—	+	+	+	—	-	-	-
	ELISA	—	+	+	+		+	+	-
	SRHD		+	+	+	—	+	+	+
Negative sera (20)	CFT	-	-	-	-	-	-	-	-
	ELISA	-	-	-		-	-	-	-
	SRHD	-	-	-	-	-	-	-	-

* + : positive results
- : negative results

The determination of the hemolysis diameter of SRHD. Table 2 shows the average hemolysis diameter which corresponds to each serum dilution (4 specemen's to each dilution), hemolysis diameter varied from 0.39 cm to 0.9 cm to detect the antibody titer of CMV from 1:80 to 1:512 hemolysis diameter, varied from 0.41 cm to 1.2 cm for the antibodies titer of rubella from 1:8 to 1:1024 the hemolysis diameter of negative sera give a maximum diameter 0.18 cm

Table(2): Determination of SRHD diameter in cm.

Antibody titer	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024
CMV	0.39	0.47	0.53	0.61	0.70	0.8	0.9	*NDH
Rubella	0.41	0.5	0.58	0.65	0.72	0.81	0.92	1.2
Negative sera	0..8	NDH	NDH	NDH	NDH	NDH	NDH	NDH

* NDH: no detectable hemolysis

Determination of IgG and IgM

Table 3 shows that average hemolysis diameter may reduced partially or completely when the sera sample was treated with 0.2m 2-ME due to presence or absence of IgM (4 specemen's to each dilution), the interpretation of the results summarized in table 4

The reduction in hemolysis diameter varied from 0.3 - 0.65 cm. in case of rubella antibodies, while in case of CMV the reduction in hemolysis diameter varied from 0.4-0.61 cm.

Table (3): The reduction in hemolysis diameter due to the action of 2-ME

Virus		Hemolysis diameter in SRHD/cm	
		Non-treated with 2 ME	Treated sera with 2 ME
CMV	1	0.39-0.9	0.39-0.9
	2	0.39-0.7	NDH
	3	0.39 0.7	0.45-0.61
Rubella	1	0.41 1.2	0.41-1.2
	2	0.41-0.8	NDH
	3	0.41-0.8	0.4 0.65

Table 3 shows that the first line of CMV and rubella hemolysis diameter shows no changes in hemolysis after and be for treatment with 2 ME, this mean the serum containing IgG only; the

second line shows complete reduction of hemolysis with the respect of the treated sera, while the third line shows partial reduction in hemolysis is the treated sera with 2ME, which mean that the sera containing both IgG and IgM, all these results confirmed by ELISA IgM test.

Interpretation of the results

Table(4): Summarized the interpretation of the result

	Treated sera with 2ME	Non-treated sera with 2ME	Type of Ig
Hemolysis in the gel	+ +	+ +	IgG
	-	+ +	IgM
		↓ ↓	IgG and IgM

* + + hemolysis
↓ reduction in hemolysis

Early diagnosis of CMV and rubella infection is very important mainly in congenital infection, specially the first months of pregnancy. In this work we compared different laboratory methods: ELISA (IgG, IgM) CFT and SRHD. After the addition of two modifications first we used sensitized RBC with tannic acid to make this test realable to all viruses and the second modification that we used 2-ME. Most of these serological tests are bast on either detection of IgG and IgM or correlation of antibodies of different activities in temporal basis with onset of the disease. The evaluation of any method should be based on many criteria, such as sensitivity, cheap and realable data presented in this paper show that SRHD detected higher antibody titer to CMV and rubella in grater proposition of specimen than did ELISA and CFT, when SRHD evaluated against CFT and ELISA (table 1) the titer determined by SRHD for 50 positive CMV samples ware three fold more than that determined by CFT and two fold more than that of ELISA (which is presumably the most sensitive test in serology) same results observed with rubella. In addition that SRHD can be determined in a single serum sample, giving accurate, specific, rapid, simple, low coast, sensitive, and SRHD technique performed without the need of special experiences and equipments.

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الخلاصة

من اهم التشخيص السكسن لاصابات الحصبة الالمانية والـCMV وخاصة في النساء الحوامل لما لهذه الفايروسات خاصية عبور المشيمة ووصولها الى الجنين مسببة تلفاً خطيراً له بأحداث تشوهات خلقية كبيرة وفي الحالات تسبب موت الجنين لذا فان وجود طريقة سهلة وحساسة ومعتمدة للكشف السريع عن هذه الفايروسات مهم جداً. أن جميع الطرق المتبعة تحتاج الى نموذجين أو ثلاثة سيرم كلا منهما اسبوع الى ثلاثة اسابيع .

لقد أجرينا تعديلات على طريقة التحلل الدموي المنتشر وذلك باستخدام كريات الدم الحمراء المحسسة بحامض التانيك لتصبح هذه الطريقة قادرة على الكشف عن جميع الفايروسات التي تسبب الخثرة الدموية والتي لا تسبب بعكس الطريقة القديمة والتي لا تستخدم هذه الطريقة الا الفيروسات القادرة على التلازن والتميع. الاكثر استخدماً 2-ME كما هو معروف عن هذا المركب الذي يفرق بين الـIgG و IgM .

بعد التطوير لهذه الطريقة يمكن استخدام نموذج واحد للمصل ليعطي نتائج مشابهة بالطرق المختبرية الاخرى وقد تم الكشف عن تراكيز الاجسام المضادة في الحصبة الالمانية من 1/8 الى 1/1024 وللـCMV 1/8 الى1/512 اي ان هذه الطريقة اكثر حساسية من الـCFT بثلاث مرات واكثر حساسية من الـELISA بمرتين والتي تعتبر أكثر الطرق المختبرية حساسية