Rubella virus and levels of Interleukin – 4 (IL-4) in Diabetic Patients in Kirkuk Governorate.

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Abstract

The present study aimed to detect the levels of (IgM and IgG) immunoglobulins and levels of Interleukin – 4 (IL-4) in their sera of women diabetic patients in Kirkuk Governorate. The study included a collection of venous blood samples from 110 women with DM in age ranged from (15-35) years . Out of 110 DM samples positive results by Immunochromatography method detected in 2 samples (1.81%) IgM only24 (21.8%) positive samples for IgG only and 1(0.9%) samples showed positive results for both IgM and IgG for rubella virus respectively .Same samples retested with ELISA for detection of IgG which revealed 22(20%) samples were positive IgG of rubella virus . The sensitivity , specificity, predictive value , accuracy rate, positive concordance rate, negative concordance rate, total concordance rate and total disconcordance rate of Immunochromatography test was 85.1% , 96.3 %, 92%, 92.6%, 28% , 64.6% , 92.68% and 7.3% respectively. Regarding Interleukin – 4 (IL-4) levels current study revealed that there was an elevation in concentration of the cytokine (IL – 4) in rubella virus infected patients serum (68.7) pg/ml when compared with control group .

Key Words: Rubella virus , Immunochromatography , Diabetics ,ELISA,

الخلاصة

فايروس الحصبة الالمانية ومستوى الانترلوكين –4 بين مرضى السكري في محافظة كركوك

هدفت الدراسة الى معرفة العلاقة بين فايروس الحصبة الالمانية ومرضى السكري في مدينة كركوك من خلال التحري عن وجود الاجسام المضادة للفايروس في مصول مرضى السكري . تم جمع 110 عينة دم من مرضى السكري بالاضافة الى 30 عينة من اشخاص لا يعانون من ا لسكري.لقد استخدمت تقنية Imminochromatography في التحري عن وجود الاجسام المضادة من نوع (IgM) و (IgG) واستخدام تقنية الالايزا للتحري عن (IgG) . واظهرت النتائج باستخدام تقنية Imminochromatography وجود (IgM) و (IgG) وكلاهما في :1.81% و 21.8% و0.9% على التوالي . لقد اظهرت الدراسة ان انتشار فايروس المضخم للخلايا البشري في مجموعة الدراسة كانت اعلى بالمقارنة بمجموعة السيطرة مما يعني ان مرضى السكري اكثر عرضة للاصابة بفايروس المضخم للخلايا البشري. نتائج دراسة الحساسية و الخصوصية لفحص Imminochromatography بالمقارنة مع فحص ELISA كانت : 85,1% و 96,3% على التوالي .فيما يتعلق بمستوى الانترلوكين –4 اظهرت النتائج وجود فروقات معنويه لمستوى الانترلوكين –4 بين المصابين بفايروس الحصبة الالمانية ومرض السكري عن غير المصابين بفايروس الحصبة لمرضى السكري .

الكلمات المفتاحية الالايزا , داء السكري , فحص Imminochromatography , فايروس المضخم للخلايا البشري.

Introduction

Rubella (German measles) [Brooks *et al* .,2007] .The rubella name is derived from Latin meaning (Little red) rubella was initially considered to be a variant of (measles) or (scarlet fever) and was called (Third disease) it was first described as a separate disease in the German medical literature .[Kathleen ,2005]. Rubella is an acute febrile illness ,which caused by rubella virus ,from Togavirus family genus Rubivirus. The disease is characterized by a rash and lymphadenopathy that affect children and adults .It is the mildest of common viral exanthemas. [Verma, 2004]. Rubella is a mild viral infection of childhood caused by non- arthropod born member of family togaviridae. At least half of all primary rubella infections are subclinical. [Gershon, 2000]. However if it is acquired during pregnancy it may cause: stillbirth, abortion, premature delivery, low birth weight and a number of congenital anomalies. [Turgut *et al*., 2004].Rubella infection during pregnancy may result in

infection of the placenta and fetus but only laminated number of fetal cells become infected that may lead to abnormalities in the number and cause abortion in the mother [Robertson *et al*., 2003]. Rubella specific IgM is diagnostic of acute infection : IgM usually appears within four days after onset of the rash and can persist up to 4-12 weeks [Bullens *et al*., 2003]. Rubella- specific IgG is a long term marker of previous rubella infection, IgG begins generally lasts for life , because of the successful immunization program in general IgM production is the acute reaction. [Banerji *et al* .,2005]

Cellular and humoral immune processes are integrally linked with each other during the initial induction of immunity to a pathogen. This disconnect between memory T cells and neutralizing Ab titers has been seen with other viral pathogens such as vaccinia virus and hepatitis B virus **[**Robertson *et al* .,2003, Pritish *et al* .,2009**]**Several broad patterns of cytokine production can be distinguished: proinflammatory cytokines such as (TNF-a or IL-6) play critical roles early in infection, Th-1 responses characterized by(IL-2 or IFN-g)drive robust cytotoxic T-cell activity, and Th-2 responses defined by the production of(IL-4, IL-5, IL-10, and IL-13) shape humoral immunity [Rolph *et al* .,2003& Smith *et al* .,1998].

Cytokines have traditionally been divided into families dependent upon the immune cell of origin and the immunological effects that they bring about. CD4+ T-helper cells are the major immune cells involved in cytokine production [Hussey and Klein *et al* .,1999] T-helper 2 cells produce IL-4, IL-5, IL-6 and IL-10, which are the main effectors of antibody-mediated humoral responses [Shohat *et al* .,2000 ,Okada Smith *et al* .,2001] by inactivating natural killer cells through HLA-G expression,[Mascola Smith .,1999 ,Elhaney Smith *et al* .,2005]. Incomplete tolerance might therefore result in disturbed pregnancy such as spontaneous abortion and pre-eclampsia [Rolph *et al* .,2003& Smith *et al* .,1998]. Releasing of cytokines at the feto-maternal interface have been proposed to play an important role in regulating embryo survival controlling not only the maternal immune response but also angiogenesis and vascular remodeling [Elhaney Smith *et al* .,2005, Shohat *et al* .,2000]. Th-1 cytokines are considered to be detrimental to pregnancy, via direct embryo toxic activity, or via damage to the placental trophoblast, or by activating cells that are deleterious to the concept us, whereas (Th-2) cytokines may directly or indirectly contribute to the success of pregnancy by down regulating potential Th-1 reactivity [Okada Smith *et al* .,2001, Mascola Smith .,1999]. Protect the fetus and placenta from being rejected and to aid in the maintenance of normal pregnancy. In humans an important role for the Th-2 immune response has also been reported during normal pregnancy**.[** Elhaney Smith *et al* .,2005**]**

Materials & Methods

Patients and control group

In this study a total of 110 DM women were selected to study the role of Rubella virus. The age of studied women ranged between (15-35). A blood sample was taken from each patient. . Thirty healthy women were selected as a control group. All samples tested by Immunochromatography test(CTK Bioteck InC: USA) and retested by ELISA test (Bio CheK ,Inc : USA) for detection IgG . Plasma samples were harvested and analyzed for cytokine by enzyme-linked immunosorbent assay techniques with commercially available kits (Cusabio : RPC). The enzyme-linked immunosorbent assay kits for interleukin (IL)-4

Collection of Blood Samples

Five ml blood samples were obtained from all studied women after cleaning the skin with 70% alcohol. Blood samples were stored in plastic tubes and left to clot undisturbed for about 1/2 hr at room temperature. Then they were centrifuged for 5 min at 3000 r.p.m(8). and then the serum was transferred into other new tubes. All serum samples were collected, stored at –20 ºC until they were tested

Results

Out of 110 DM samples samples positive results by Immunochromatography method detected in 2 samples 1.81%IgM only 24 samples 21.8% IgG only and 1(0.9%) sample showed positive results for both IgM and IgG for rubella virus Table 1. In this study ,serum samples from 110 patients with DM , which tested by Immunochromatography for rubella virus were retested with ELISA for detectin IgG which revealed 22(20%) IgG were positive of rubella virus Table 2 . The sensitivity , specificity, positive predictive value , accuracy rate, positive concordance rate, negative concordance rate, total concordance rate and total disconcordance rate of Immunochromatography test was 91.6% , 95.3 %, 84.6%, 94.5%, 20% , 74.5% , 95.5% and 5.45% respectively …Table 3 . Regarding Interleukin – 4 (IL-4) levels revealed that there was an elevation in concentration of the studied cytokine (IL – 4) in rubella virus infected DM patients serum (mean: 102.4) pg/ml in comparasion with non infected with rubella virus DM patients and control group with a highly significant differences ($p < 0.01$)…Table 4

Table 1:Results of IgM and IgG of rubella virus among DM patients .

Type of tested samples	Results of Immunochromatography .											
	IgM				IgG				IgM and IgG			
	Positve		Negative		Positve		Negative		Positve		Negative	
	No.	%	No	%	No	%	No	%	No	%	No	%
DM samples	2	1.81	108	98.1	24	21.8	86	78.1	1	0.9	109	99.0
Control samples	0	0	30	100	11	10	100	90	0	0	110	100

Table 2 .Comparison of Immunochromatography and ELISA Tests for detection of IgG of rubella virus in 110 Specimens from DM women cases .

Test	Tested Cases	+ve		-ve	
		No.	%	No.	%
ICT Test*	110	24	21.8	86	78.1
ELISA Test	110	22	20.0	90	80

* ICT Test: Immunochromatography Test

Table 3 :Evaluation of Immunochromatography Test Validity as Compared with ELISA Test for Detection of IgG of rubella virus in 110 Specimens from DM women cases .

ELISA / *ICT	Positive results	Negative results	Total
Positive results	**22**	**4**	**26**
Negative results	**2**	**82**	**84**
Total	24	86	110

* ICT Test: Immunochromatography Test

$$\text{Sensitivity of latex test} = \frac{22}{24} \times 100 = 91.6\ \%$$

$$\text{Specificity of latex test} = \frac{82}{86} \times 100 = 95.3\ \%$$

$$\text{Positive predictive value of latex test} = \frac{22}{26} \times 100 = 84.6\ \%$$

$$\text{Accuracy rate} = \frac{22+82}{110} \times 100 = 94.5\%$$

$$\text{Positive concordance rate = agreement in positivity of PCR and latex} = \frac{22}{110} \times 100 = 20\ \%$$

$$\text{Negative concordance rate} = \frac{82}{110} \times 100 = 74.5\ \%$$

$$\text{Total concordance rate} = \frac{22+82}{110} \times 100 = 95.5\%$$

$$\text{Total disconcordance rate} = \frac{2+4}{110} \times 100 = 5.45\ \%$$

Table 4: Comparison of serum level (mean ±SE) of IL-4 in DM with Rubella positive DM patients , Rubella Negative non- DM patients and control group

Studied Groups	Number of Samples	Range	Mean (Pg/Ml)	SD±
Rubella +Ve DM Cases	**22**	**87.5-123.7**	**92.3**	**25.2**
Rubella -Ve DM Cases	**15**	**44.5-68.7**	**59.0**	**13.9**
Control	**15**	**20.3-27.7**	**24.4**	**20.1**

SD. = Standard deviation S.E. = Standard Error, DM =Diabetics Mellitus

Discussion

The aim of all rubella virus antibody detection techniques is to get maximum sensitivity with specificity. When large volumes of samples are to be tested, a technique that is simple and fast to perform giving results that are more reliable, easily interpreted and cost effective is required .In our study IgG rubella ELISA showed (91.6%) sensitivity and(95.3%) specificity respectively, this is comparable to Wittenburg *et al* who have shown (61.7%) sensitivity & (95%)specificity in their study [Rebecca *et al* .,1985] . In a study by Field *et al*, who evaluated 3 rubella ELISA kits, showed the following results/RUBELISA showed a sensitivity of 95.6% & a specificity of 97%, Enzygost-Rubella (99.26% , 100%) and Ortho rubella (100% & 97.32%)respectively.In a study similar to ours, Terada K *et al* have compared ICT assay with ELISA technique for detection of IgG antibodies against rubella. ELISA technique showed (100%)sensitivity & specificity, and ICT showed (99.35%) and (100%) [Torada *et al* .,2000].Wu Jian Mei *et al* have used An IgM ICT in detecting IgM antibodies to TORCH. They found 100% sensitivity and specificity with ICT[7]. In an epidemiological investigation of rubella virus by Mu Ying *et al*, they mentioned that IgG ICT was a highly sensitive and specific test [Wu Jin-Xiang *et al* .,2008] ELISA-technique continues to be the gold standard for detection of immunity against rubella virus. Though it is highly sensitive and specific, it is not easy to perform, requires trained technical personnel and reports have long turnaround time. On the other hand, ICT assay is a rapid test has been added to the battery of available rubella tests. This test unlike ELISA technique requires no pre -treatment of sera, elaborate equipment and can be performed in a matter of minutes. If ICT had high inbuilt sensitivity and specificity controls, as shown by other authors, it could be an acceptable alternative to ELISA technique . ICT could very well be most useful for clinical laboratories performing tests for where immediate results are required for management of patients.The role of cellular immunity in rubella is as yet poorly defined. One of the goals of this study was to more clearly characterize cell-mediated immune responses to rubella virus, and to determine potential associations between humoral and cellular immune responses to rubella vaccination.The immune response to virus infections and attenuated virus vaccines seems to depend on various factors including genetics, age and sex. Thus, measles patients of 1_/2 years of age are least severe in clinical symptoms and lymphopaenia, whereas young infants before their first birthday as well as adolescents and adults will progress to severe conditions.10 Studies from Guinea-Bissau and South Africa have observed increased mortality due to measles in girls,[Elhaney Smith *et al* .,2005] and girls are more likely to develop fever and rash after routine measles_/mumps_/rubella immunization [Mitchell *et al*.,1999]. Similarly to measles vaccine recipients.In contrast to the rubella patients demonstrating at ransient increase in IL-10 level, a sustained elevation of the cytokine concentration in rubella vaccine recipients has been found. The IL-10 levels increased as early as day 7 and amounted to a maximum on day 30. Simultaneously, a significant reduction in plasma IFN g and a profound decrease of peripheral blood lymphocyte response to PHA have been shown. These changes were accompanied with marked elevation of IL-4. It may be proposed that the observed cytokine 'shift' from type 1 cytokines early after vaccine inoculation to type 2 cytokines 1 month after vaccination is associated with activation of central mechanisms of immunosuppression [Chan *et al* .,2013].In current study Interleukin – 4 (IL-4) levels revealed that there was an increasing in concentration of the studied cytokine (IL – 4) in rubella virus infected DM patients serum (mean: 102.4) pg/ml in comparasion with

non infected with rubella virus DM patients and control group with a highly significant differences ($p < 0.01$). **[**Kennneth *et al* .,1995**]**

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