Study of the Relationship between Some Microorganism Isolated from Congenital Anomalies Neonatal Screening and Their Mothers in Iraqi Patient

دراسة العلاقة بين بعض الاحياء المجهرية المعزولة من الاطفال المصابين بالتشوهات الخلقية الولادية وامهاتهم من المصابين العراقيين

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المستخلص:

الهدف : سعت هذه الدراسة الى تحديد IgM و IgG في مصل الامهات واولادهم من الاطفال الذين يعانون تشوهات خلقية من المرضى العراقيين اتجاه عدة فايروسات وهي التوكسوبلازما وفايروس الحصبة الالمانية وفايروس الهربس البسيط وفايروس المظخم للخلايا وعلاقتها مع العمر للام والابن وجنس الاطفال وجمعت العينات من مستشفى العلوية للاطفال .

المنهجية : ٥٠ عينه دم جمعت من الامهات واطفالهم المصابين بالتشوهات الخلقية الولادي للكشف عن IgM و IgG لبعض الاحياء المجهرية وهي طفيلي التوكسوبلازما وفايروس الحصبة الالمانية وفايروس الهربس البسيط وفايروس المظخم للخلايا وشخصت بطريقة فحص الانزيم المناعي الاليزا .

النتائج : الدراسة الحالية اظهرت ٢٧ ذكر و ٢٣ انثى واعمار الامهات كانت ما بين ١٨– ٣٣ اما الابناء من ١ يوم – ١٨ شهر معدل متوسط الدراسة ٢٤,٤٦ مع والانحراف المعياري ٤,٣٥ . لوحظ في الدراسة الحالية بان IgG للتوكسوبلازما كان مستواه عالي عند االامهات اكثر من الابناء عند ٣٧ و ٢٠ (٧٤ و ٢٠) % غلى التوالي كنتيجة موجبة وكان اعلى تردد IgGللمرض عند الامهات اكثر من IgG عند الاطفال الذكور ١٩ و ١٠ (٧٠,٣٧و ٣٧,٠٤) % على التوالي و ١٨ و١٠ (٧٨,٢٦ و ٤٣,٤٨) % على التوالي عند الاطفال الاناث . وكذلك كانت نتيجة IgG موجبة للفايروس المظخم للخلايا للاطغال ٣ (٦%) بتوزيع ١(ذكر ٢ انثى) كنتيجة موجبة و IgM للفايروس عند الام ٣ ذكر و٢ انثى (١١,١١ و ٨,٧) % على التوالي . من جهة اخرى ارتفاع IgM لفايروس الهربس البسيط للاطفال كان ٣ (٦%) كنتيجة موجبة بينما انثى واحدة و٢ ذكر (٧,٤١و ٤,٣٥)% على التوالي واخيرا النتيجة الموجبة للمصابين بفايروس الحصبة الالمانية كان ٣(٦%) لل IgM للاطفال وزعت ١ انثى و٢ ذكر (٧,٤١ و ٤,٣٥) % على التوالي .

التوصيات: توعية الوالدين حول الامراض المنتقلة جنسيا وخصوصا حول الاحياء المجهرية المسببة لها كذلك اوصي بالدراسات الجزيئية لتشخيص ومعرفة دور هذه الاحياء المجهرية المسببة للامراض الجنسية لكل من الام وطفلها .

Abstract

Objectives : This study was seeks to determine the IgG and IgM in serum mothers and their babies of Iraqi patient suffering from congenital anomalies toward some microorganisms such as Cytomegalovirus (CMV), Congenital toxoplasmosis , Congenital rubella and Genital herpes simplex virus (HSV) correlated with age and babies gender the sample was collected from AL- Alwayia hospital for children / Baghdad .

Methodology : Fifth blood sample have been collected from mothers and their babies suffering from congenital anomalies to detection IgG and IgM of some viruses including as Cytomegalovirus (CMV), congenital toxoplasmosis , Congenital rubella and Genital herpes simplex virus (HSV) was diagnosed by , enzyme-linked immunosorbent assay ELISA .

Results : This study was showed that 27 male and 23 female , the mother's age was 18-33 years old while babies' s age was 1 day – 18 months , the mean of this study was 24.46 with Std 4. 35 . The current study was noted the IgG of Congenital Toxoplasmosis in serum of mothers more than their infant at 37 , 20 (74 , 40) % respectively as positive result , also the highest frequency for the disease was IgG in mothers more than IgG in male babies of 19, 10 (70.37 , 37.04) respectively and 18 , 10 (78.26 , 43.48) respectively in female babies , also the high level of IgM of CMV in babies was 3 (6 %) (1 male and 2 female)of positive result distributed as IgM in mothers and baby at 3, 2 (11.11 , 8.7)% respectively , on the other hand the IgM of HSV in babies was 3 (6%) positive result 2 (7.41 %) positive result with male babies while 1 (4.35 %) with female baby. Finally the positive result of Rubella infection was with IgM babies at 3(6%) distributed the positive result was 2 (7.41%) in male babies and 1 (4.35 %) in female baby.

Key words : Congenital Anomalies , HSV , CMV , Toxoplasma , Rubella

Introduction

A congenital anomalies, is a disorder present in before birth depend on it causes according to change in a developing fetus called teratogen that mean reason birth defect ; prenatal diagnosis screening test used for detect some these disorders before birth [1]fetal infection of congenital anomalies was show presence of disease by measure IgM antibody immediately after birth or determination of IgG after the first year [2] , There are many microorganisms that cause infection with congenital anomalies such as ; Cytomegalovirus (CMV) [3] ; this virus is a ds DNA , contains numerous virally encoded glycoproteins in the outer envelope of this virus will be resulting from the nuclear membrane of host cell, [4] The other one is congenital toxoplasmosis toxoplasmosis (CT) that regarded as abroad range of medical sign at birth, as well as ; neurological variable degree , ophthalmological and systemic participation [5] , on the other hand , asymptomatic toxoplasmosis appear in infant but convert to symptoms from numerous weeks to some months reason transmission of Toxoptasma gondii from mother s disease to her fetus by placenta during pregnancy , or by oral and by either of the two forms of infection ; cysts in the infected animal and oocysts in the feces of an infected cat [6].While Genital herpes simplex virus (HSV) infections is recurrent sexual disease between the mature women of the United States and often asymptomatic or undiagnosed, [7] Nonetheless there are some feature associated with this virus like pressure, discomfiture, the late-pregnancy acquisition of genital infection is much risk in newborn disease ; repeated infections are seldom associated with spread neonataldisease; The latent stage HSV types 1 (HSV-1) and 2 (HSV-2) will migrate to nerve tissues by transmitted of virus throughout epithelial mucosal cells via skin interruptions, [8] . Finally , the last type of infection in this study is Congenital rubella (CR), an essential source of severe birth defects, remains a public health problem due to the teratogenic effects and risk of miscarriage and stillbirth that may result from congenital infection, there are some cases undiagnosed or misdiagnosed with subclinical Rubella infection is frequently minor withnonspe-cificsymptoms.[9-11].

Methodology

The cross sectional study was design in this research . A total of 50 samples form mothers and 50 blood samples from their babies of congenital anomalies , (27male to 23 female) , the patients were appearing the AL- Alwayia hospital for children in Baghdad through the dated from 3 /2015 to 8 / 2015.The mother's age was 18 – 33 years old and baby's age was 1 day – 18 month .

Sample collection : Three ml of blood was collected from each patient and then serum was separated and distributed into three eppendorff tubes for ELISA methods and then kept it in freez at -20 °C . The detection of IgM and IgG from mothers and their infant by serological as rapid test . Frequently this diagnoses is important to be differentiate between a acute and a chronic infection, IgM and IgG of viral infection with congenital anomalies was diagnosis by enzyme-

linked immunosorbent assay ELISA (Human , Germany). Firstly Patient's sera was diluted 1 to 100 µl in sample diluent ; 100 µL of serum samples per well was added to antibody coated wells and incubated for 1 h at 37 C , the wells was washed by washing buffer four time ; and then100 µL of Toxoplasma conjugate also was added mix carefully for 5 sec and plate was covered with cover plate and then incubated for 30 sec at 37 C, with shaking , the wells was washed again four time .Finally 200µL of TMB substrate was added to each wells and then incubated for 30 min at room temperature in the dark, with shaking , The reaction was terminated after add 100 µL of stop solution into the wells , The well was measured at 450 nm. The same procedure was used for detection IgM of Rubella , CMV and HSV except the incubation time after added sample serum was 1 h at room temperature Estimation of IgG of the same viral infection (Human , Germany) for mothers and their babies By ELISA test for the qualitative detection dependent of the producer's protocol . One hundred µl of ; Diluent sample was added then incubated for 30 min at room temperature , the wells was washed by washing buffer four time ; then100 µL of Toxoplasma anti- IgG conjugate also was added. Then the plate was covered and incubated for 30 min .at room temperature with shaking , the wells was washed again for five times . One hundred µL of TMB substrate was added to each wells and then incubated for 25 min at room temp. in the dark, with shaking. The reaction was terminated by adding 100 µL of stop solution into the wells. The absorbance was measured at 450 nm. The same procedure was used for detection of IgG of Rubella , CMV and HSV . SPSS statistics 18 version was used for Analysis of data . Mean value, Standard Deviation was found , P value < 0.05 was measured statistically significant.

Results

Table (1) :Distribution of Samples According to Number and Percentage of Congenital Toxoplasma between Mothers and Their Infant

Toxoplasma		No	Percentage
IgM Baby	negative	49	98.00 %
	positive	1	2.00 %
IgM Mother	negative	50	100.00 %
	positive	0	.00
IgG Baby	negative	30	60.00 %
	positive	20	40.00 %
IgG Mother	positive	37	74.00 %
	negative	13	26.00 %
	Total	50	100.00 %

IgG : Immunoglobuline G , IgM : Immunoglobuline M , No : number

This table shows that IgG of Congenital Toxoplasmosis in serum of mothers more than their infant at 37 , 20 (74 , 40) % respectively as positive result , while one (2%) positive result of IgM from infant and not appear in mothers .

Table(2): Distribution of Congenital Toxoplasmosis According to Gender of Babies

Toxoplasma		Gender						
		male		Female		Total		P.V
		N0	%	N0	%	N0	%	
IgM Baby	negative	27	100,00	22	95,65	49	98,00	0.274
	positive	0	,00	1	4,35	1	2,00	
IgM Mother	negative	27	100,00	23	100,00	50	100,00	-
	positave	0	,00	0	,00	0	,00	
IgG Baby	negative	17	62,96	13	56,52	30	60,00	0.643
	positive	10	37,04	10	43,48	20	40,00	
IgG Mother	positive	19	70,37	18	78,26	37	74,00	0.526
	negative	8	29,63	5	21,74	13	26,00	
	Total	27	100,00	23	100,00	50	100,00	

IgG : Immunoglobuline G , IgM : Immunoglobuline M , P.V : P value significant ≤0.05

The current study confirms that the maximum frequency for this disease was IgG in mothers more than IgG in male babies of 19, 10 (70.37 , 37.04) % respectively and 18 , 10 (78.26 , 43.48) % respectively in female babies , on the other hand this study appear one IgM positive result in female baby at (4.35)% , there are no statistical significant between it.

Table (3) : Number and Frequency of CMV for Both Mothers and Baby

CMV		No	percentage
IgM Baby	negative	47	94.00 %
	positive	3	6.00 %
IgM Mother	negative	45	90.00 %
	positive	5	10.00 %
IgG Baby	Negative	50	100.00 %
	positive	0	.00
IgG Mother	negative	50	100.00 %
	positive	0	.00
	Total	50	100.00 %

CMV: Cytomegalovirus , No : number , IgG : Immunoglobuline G , IgM : Immunoglobuline M

Number and Frequency of CMV for both mothers and baby in this table shows that 3 (6 %) of positive result with IgM babies was appeared in this study , while negative result appear for the other baby and mother .

Table (4) : Distribution of Congenital CMV According to Mother Associated with Babies Gender

CMV		Gender of babies						
		male		female		Total		P.V
		No	%	No	%	No	%	
IgM Baby	negative	26	96,30	21	91,30	47	94,00	0.459
	positive	1	3,70	2	8,70	3	6,00	
	Total	27	100,00	23	100,00	50	100,00	
IgM Mother	negative	24	88,89	21	91,30	45	90,00	0.777
	positive	3	11,11	2	8,70	5	10,00	
	Total	27	100,00	23	100,00	50	100,00	
IgG Baby	negative	27	100,00	23	100,00	50	100,00	-
	positive	0	,00	0	,00	0	,00	
	Total	27	100,00	23	100,00	50	100,00	
IgG Mother	negative	27	100,00	23	100,00	50	100,00	-
	positive	0	,00	0	,00	0	,00	
	Total	27	100,00	23	100,00	50	100,00	

CMV: Cytomegalovirus , No : number , IgG : Immunoglobuline G , IgM : Immunoglobuline M
P.V : P value : significant ≤ 0,05

Distribution of congenital CMV according to mother associated with babies gender appears in this table showed that no statistical significant appear with correlation between CMV and babies gender , the result appeared positive result for IgM in mothers and baby at 3, 2 (11.11 , 8.7)% respectively and negative result with IgG for both mothers and babies .

Table (5): Number and Percentage of HSV Finding for Mothers and Baby

HSV		No	percentage
IgM Baby	negative	47	94.00 %
	positive	3	6.00 %
	Total	50	100.00 %
IgM Mother	negative	50	100.00 %
	positive	0	.00
	Total	50	100.00 %
IgG Baby	negative	50	100.00 %
	positive	0	.00
	Total	50	100.00 %
IgG Mother	negative	50	100.00 %
	positive	0	.00
	Total	50	100.00 %

HSV : Herpes simplex virus , No : number , IgG : Immunoglobuline G , IgM : Immunoglobuline M

The result in this table shows that 3 (6%) positive result was appeared with IgM babies , while the negative result noted for the others.

Table (6) : Distribution of HSV Finding Correlated Between Mothers and Babies Gender

HSV		Gender						
		male		female		Total		P,V
		No	%	No	%	No	%	
IgM Baby	negative	25	92,59	22	95,65	47	94,00	0.650
	positive	2	7,41	1	4,35	3	6,00	
	Total	27	100,00	23	100,00	50	100,00	
IgM Mother	negative	27	100,00	23	100,00	50	100,00	-
	positive	0	,00	0	,00	0	,00	
	Total	27	100,00	23	100,00	50	100,00	
IgG Baby	negative	27	100,00	23	100,00	50	100,00	-
	positive	0	,00	0	,00	0	,00	
	Total	27	100,00	23	100,00	50	100,00	
IgG Mother	negative	27	100,00	23	100,00	50	100,00	-
	positive	0	,00	0	,00	0	,00	
	Total	27	100,00	23	100,00	50	100,00	

HSV : Herpes simplex virus , No : number , IgG : Immunoglobuline G , IgM : Immunoglobuline M , P.V : P value significant ≤ 0.05

Distribution of HSV finding correlated between mothers and babies gender is presents in table (6) has revealed no statistical significant in this study , this study appeared that 2 (7.41 %) positive result with male babies while 1 (4.35 %) with female baby and negative result for others .

Table (7) : Number and Percentage of Rubella Finding for Mothers and Baby

Rubella		No	percentage
IgM Baby	negative	47	94.00
	positive	3	6.00
IgM Mother	negative	50	100.00
	positive	0	.00
IgG Baby	negative	50	100.00
	positive	0	.00
IgG Mother	negative	50	100.00
	positive	0	.00
	Total	50	100.00

No : number , IgG : Immunoglobuline G , IgM : Immunoglobuline M

Number and percentage of Rubella finding for mothers and baby have revealed positive result of Rubella infection was with IgM babies at 3(6%) but other test was negative result for all .

Table (8) : Distribution of Congenital Rubella According to Mothers Correlated with Babies Gender

Rubella		Gender						
		male		female		Total		
		No	%	No	%	No	%	P.V
IgM Baby	negative	25	92.59	22	95.65	47	94.00	0.650
	positive	2	7.41	1	4.35	3	6.00	
IgM Mother	negative	27	100.00	23	100.00	50	100.00	
	positive	0	.00	0	.00	0	.00	
IgG Baby	negative	27	100.00	23	100.00	50	100.00	
	positive	0	.00	0	.00	0	.00	
IgG Mother	negative	27	100.00	23	100.00	50	100.00	
	positive	0	.00	0	.00	0	.00	
	Total	27	100.00	23	100.00	50	100.00	

No : Number , IgG : Immunoglobuline G , IgM : Immunoglobuline M , P.V : P value significant ≤ 0.05

Distribution of congenital Rubella according to mothers correlated with babies gender is show in this table there were no statistical significant with Rubella infection correlated with babies gender, the positive result was 2 (7.41%) in male babies and 1 (4.35 %) in female baby but the other test was negative result .

Discussion

The IgG of Congenital Toxoplasmosis in serum of mothers was found more than in infant while one positive result of IgM from infant and not appear in mothers , this may be due to IgM antibody titers get higher from ; 5 days - weeks subsequent severe infection, become a supreme infected after 1 - 2 months and deterioration further quickly than IgG [12] ; In acute diagnosis of Toxoplasmosis , IgM antibody is appear in serum of patient but cannot always indicator for infection , usually the IgG antibody appear after IgM antibody and can be detect after 1-2 week and sometime after more than 12week to 6 months after acute infection; On the other hand , the lack of infection or tremendously new acute infection is appear during IgG and IgM both negative[13] .

When the positive IgG and negative IgM is appear,this indicate the old infection more than one year ago, if both IgG and IgM are positive this mean a recent infection or a false-positive test [14] also finding of IgM and/or IgA in serum of babies ; are approximately probable to guess the real occurrence of congenital toxoplasma because some of affected newborns are not presence during neonatal screening, which may not notice [15].The presence of CMV IgM in serum of baby indicate congenital

infection due to motherly IgM antibodies can't cross the placenta to the baby . On the other hand ; the sensitivity's detection of CMV antigen in blood is low when the speedy diagnosis may be gotten. If the result was IgM antibodies in the newborn's serum that indicative of congenital infection but IgG antibodies in neonates was appear this indicate mostly maternally transferred antibodies[16], The number and percentage of HSV for mothers and baby was found positive result with IgM babies , this result may be due to the medical marks of CMV and HSV acquired from the placenta is begin to seem after tenth day of lifetime. [17] , negative result of HSV virus in mothers may be due to that HSV IgM is uncommonly initiate in adults, subsequently greatest person obtain HSV in juvenile but HSV IgM is raised in all patients [18] on the other hand ; pregnant women there is a grade of physiological immunosuppression in different may be causes activation of infection by opportunistic agents [18]also the viral reactivation becomes more likely ; when increased risk of obstetrical and maternal damage [19] , so The positive result of HSV babies male at age 5 month and 23 days while female at age 13 days due to the HSV virus is not noticeable through the first three days of infant's delivery but later may be create in blood and in some body fluid. Therefore , the elevated of specific IgM levels are detectable from 15 days after birth but not constant [17] .

Distribution of congenital Rubella according to mothers correlated with babies gender was appear the infants with congenital rubella infection may have levels of rubella-specific fetal IgM and elevated levels of fetal IgG and IgA at birth [20 , 21], as well as high levels of maternally derived IgG. The interaction of antibodies with complement is probably reduced compared with adults since the concentration of complement components increases slowly from midgestation to about 50% of adult values by birth [22].

Recommendations : Awareness of each parents about sexually transmitted disease especially about the microorganisms that causes this disease also I recommend for molecular studies about the diagnosis of this microorganisms and the role of it inboth mother and their baby .

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