تحري أضداد فيروس الحصبة الألمانية من نوع IgG، لدى مجموعة من الإناث غير المتزوجات، بعمر الخصوبة من محافظتي دمشق ودرعا

عبد الغني العقاد*

الملخص

خلفية البحث وهدفه: تقدير خطر التعرض للإصابة بفيروس الحصبة الألمانية لدى الإناث اللواتي في عمر الخصوبة في جنوب سورية، الذي يعكس إمكانية تطور الحصبة الألمانية الخلقية عند حدوث حمل مستقبلي، وكذلك سبر إمكانية وجود أي ارتباط بين العمر أو الموقع الجعرافي، وعيار أضداد الحصبة الألمانية.

مواد البحث وطرائقه: تم التحري عن أضداد فيروس الحصبة الألمانية من النوع IgG في مصول مجموعة (141) من الإناث غير المتزوجات (18–25 سنة) اللواتي بعمر الخصوبة من محافظتي دمشق ودرعا، باستخدام مقايسة الممتز المناعي المرتبط بالأنزيم.

النتائج: إن 8% من الإناث اللواتي بعمر الخصوبة من محافطتي دمشق ودرعا، المشمولات بالدراسة، لا يملكن عياراً واقياً من أضداد الحصبة الألمانية، وهن معرضات لخطر إصابة مستقبلية بفيروس الحصبة الألمانية في أثناء حملهن، واحتمال حدوث متلازمة الحصبة الألمانية الخلقية. كذلك لم يتم إثبات أي ارتباط بين عمر الإناث أو مكان إقامتهن، وعيار أضداد الحصبة الألمانية لديهن.

الاستنتاج: أظهرت الدراسة المقدمة أهمية وجود نظام تقصي وبائي مستمر لتقييم مدى كفاءة برامج التلقيح ضد فيروس الحصبة الألمانية في سورية، وأهمية توحيد الطرائق المخبرية المستخدمة لتحديد المناعة تجاه فيروس الحصبة الألمانية، و كذلك الحاجة لإجراء تلقيح داعم ضد فيروس الحصبة الألمانية للإناث في سن البلوغ، لتخفيض نسبة حدوث متلازمة الحصبة الألمانية الخلقية.

كلمات مفتاحية: فيروس الحصبة الألمانية، أضداد الحصبة الألمانية من النوع IgG، متلازمة الحصبة الألمانية الخلقية، مقايسة الممتز المناعي المرتبط بالأنزيم، منظمة الصحة العالمية.

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Screening for Anti-Rubella Virus IgG Antibodies in Sera of Unmarried Childbearing Age Females from Damascus, aAnd Daraa Governorates

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Abstract

Background & Objective: It is to estimate the actual risk of exposure to Rubella virus infection in susceptible childbearing age females, in south Syria, reflecting the potential of developing Congenital Rubella Syndrome at future pregnancy, and to explore the existence of any epidemiological association between age, or geographic area, and titer of anti-Rubella antibodies.

Methods & Materials: A cohort of 141 unmarried childbearing age females (18-25 years old), from Damascus and Daraa governorates, was subjected to screening for, and titration of anti-Rubella IgG antibodies in their sera by enzyme linked immunosorbant assay.

Results: 8% of childbearing age females form Damascus and Daraa governorates enrolled in this study hadn't adequate protective anti-Rubella antibodies titers, and they might be at risk of later natural Rubella virus infection at pregnancy. Also an association between age, or geographic area, of these females, and their obtained anti-Rubella antibodies titers could not be proved.

Conclusion: The presented study demonstrated the importance of continuous surveillance system for evaluating the efficiency of enrolled Rubella vaccination programs in Syria, and the importance of harmonization of assays used for determination of immunity against Rubella virus. Also the need for introducing a mandatory booster dose of Rubella vaccine to pubescent females, to reduce the incidence of Congenital Rubella Syndrome.

Keywords: Rubella virus; anti-Rubella IgG antibodies; Congenital Rubella Syndrome (CRS); Enzyme-Linked Immunosorbant Assay (ELISA); World Health Organization (WHO).

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Introduction

Rubella, known as German measles, is a self-limited, RNA-virus induced childhood disease, manifesting, as lymphoadenopathy, and characteristic fine maculo-papular exanthema, and resolving usually without clinical complications. Yet Rubella virus infection of childbearing aged females, represents a serious risk for developing Congenital Rubella Syndrome (CRS), affecting many developmental stages of fetus، leading to variety of clinical manifestations including stillbirth, miscarriage, cataract, blindness, deafness, retardation, and cardiac defects[1,2,3,4,5,6]. Globally, the number of reported Rubella and CRS cases, is markedly declined in the recent years[7], since the implementation of national childhood MMR (Mumps, Measles, and Rubella) vaccination programs[7,8,9]. Nevertheless, estimating the actual risk of exposure to Rubella virus in susceptible childbearing age women, which may reflect indirectly the efficiency of vaccination coverage, is not clear. if Rubella's children vaccination programs were not optimally implemented, to maintain a herd immunity of 80% and higher, then a paradoxical increase in CRS can occur due to a decreased circulation of the virus and an accumulation of Rubella susceptible adult females[10]. Trying to answer these questions, we aimed to estimate the prevalence of unmarried childbearing age women, from south Syria, who are still susceptible to Rubella virus infection, due to absence of protective anti-Rubella virus IgG antibodies in their sera. A cohort of unmarried childbearing females, from Damascus, Daraa and their rurals governorates, has been chosen, and subjected to screening for, and titration of anti-Rubella IgG antibodies in their sera using Enzyme linked Immunosorbant assay technique (ELISA)[11,12].

Materials

-141 unmarried childbearing age females derived sera, from Arab international University (AIU)

-8 well snap-off strips of The {HUMAN RUBELLA lgG Enzyme linked immunoassays (ELISA) ®} diagnostic kit, coated with purified Rubella virus (RV) antigens.

-Rubela IgG negative control

-Rubella IgG Cut-off control serum (titer 15 IU/ml), calibrated against the 2nd international standard preparation (WHO anti-Rubella serum).

-Rubella IgG positive control serum (titer 100 IU/ml), calibrated against the 2nd international standard preparation (WHO anti-Rubella serum)

-Diluent buffer IgG (Phosphate buffer 10 mmol/l, NaCl 8g/l, Albumin 10g/l; PH=6.5)

-Rabbit anti-human IgG, peroxidase labeled antibodies

-Wash solution stock (Tris buffer 10 mmol/l, NaCl 8 g/l; PH= 7.2)

-Color development substrate solution (3, 3', 5, 5'-Tetramethylbenzidin (TMB) 1.2 mmol/l,

Hydrogen peroxide 3 mmol/l; PH=3.7)

-Color development stop solution (Sulphuric acid 0.5 mol/l)

-96 well format ELISA microtiter plate reader (Sunstik)® at reading wave of 450 nm, reference reading wave of 630nm

Methods

1-Selection of target study

A total of 141 unmarried childbearing aged females between 18-25 years (64% students, 29% employees, 7% technical/ teaching staff) studying or working at The Arab international University (AIU) were included in this study. These females were distributed geographically between Damascus, Rural Damascus, and Daraa governorates (39% from Damascus city; 14% from Daraa city; 15% from Rural Damascus governorate and 32% from Rural Daraa (2% from Der Yaget, 29% Gabageb, 15% Kheel, 18% Jbab and 16% Sanamain)).

2-Obtaining and processing of sera

3 ml of whole blood samples, drawn from the selected 141 females, were collected, into clean blood dry tubes (No anticoagulant additive). Following complete blood coagulation, tubes were centrifuged for 15min at a speed of 800 rpm, and separated sera were transferred into 2ml Eppendorf tubes then freeze-stored at -20 Cº till time of assay

3-Measurements of rubella IgG antibodies:

The {HUMAN RUBELLA lgG Enzyme linked immunoassays (ELISA) ®} diagnostic kit was used to screen, and quantify the previously mentioned obtained sera of unmarried childbearing aged women for anti-Rubella virus IgG antibodies according to manufacturer instructions. Briefly micro titer strip wells, coated with purified Rubella virus antigens (RV-Ag), were incubated for 30 minutes with 100 µl of females derived sera, negative control-, cut-off control-, and positive control- sera respectively, at 25Cº. Unbound components were washed out 4 times with 350 µl wash solution, followed by reincubation for 30 minutes with 100 µl of Rabbit anti-human IgG peroxidase labeled antibodies at 25Cº. Strips were rewashed 5 times with 350 µl wash solution, followed by incubation for 15 minutes with 100 µl of color development substrate solution. Finally 100 µl of color development stop solution was added to strips, and Absorbance of developed color was read at both λ=450 nm, 630 nm respectively by ELISA microtiter plate reader. Positive, cut-off, and negative control sera were used to construct a calibration assay curve

of (0, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 IU/ml) respectively of anti-Rubella virus IgG antibodies, where diluted positive control-, cut-off- and negative control sera were assayed 2 times and the arithmetic mean was obtained. Mean values were subtracted from blank values, and then Absorbance was plotted against the corresponding anti-Rubella virus IgG antibodies. Next, female sera samples were assayed according to the calibration curve.

Results

In an attempt to estimate the prevalence of anti-Rubella virus IgG antibodies in syrian females, who reached the age of childbearing, and lived in Damascus, and Daraa governorates, and to answer whether titers of these antibodies were adequate to protect them against the risk of developing CRS at future pregnancy, A total of 141 unmarried childbearing age females studying or working at The Arab international University (AIU) (39% from Damascus city; 14% from Daraa city; 15% from Rural Damascus governorate، and 32% from Rural Daraa (2% from Der yaget; 29% Gabageb; 15% Kheel; 18% Jbab; and 16% Sanamain)) were chosen to assay their sera for anti-Rubella virus IgG antibodies using the sensitive ELISA technique. These results showed that 8% (11/141) of selected females: (Sera numbers: 7; 9; 12; 20; 28; 61; 69; 75; 82; 105; 112, with anti-Rubella IgG titers of: 1; 1; 2; 6; 0; 0; 0; 13.5; 12; 14; 16 IU/ml, respectively) were seronegative, or hadn't adequate anti-Rubella virus IgG antibodies, as shown in table-1.

Table-1: ELISA assayed anti-Rubella virus IgG antibodies titers in 141 unmarried childbearing age females from Damascus and Daraa governorates.

Con; A, denote: Concentration/Titer of anti-Rubella IgG antibodies in IU/ml; and Absorbance at 340nm respectively. Titers were classified into: very low: less than 12 IU/ml (red colored fields); insufficient: between 12 to 17 IU/ml (pink colored fields); normal: above 17 IU/ml (blue colored fields); and very high: above 100 IU/ml (green colored fields) respectively.

Samples Nr.	*Con.*	*A*	*Samples Nr.*	*Con.*	*A*	*Samples Nr.*	*Con.*	*A*	*Samples Nr.*	*Con.*	*A*
1	97	2.41	32	115	2.846	63	110	2.735	94	51	1.415
2	110	2.722	33	42	1.188	64	58	1.588	95	59	1.622
3	144	3.532	34	24	0.719	65	78	2.1	96	48	1.325
4	108	2.69	35	101	2.464	66	35	1.001	97	64	1.756
5	72	1.945	36	102	2.449	67	107	2.663	98	70	1.911
6	115	2.836	37	103	2.482	68	33	0.938	99	28	0.801
7	1	0.057	38	56	1.549	69	0	0.025	100	36	1.018
8	71	1.933	39	54	1.499	70	68	1.839	101	73.5	1.994
9	1	0.025	40	103	2.566	71	40	1.141	102	79	2.127
10	161	3.913	41	53	1.521	72	40	1.146	103	69	1.873
11	82	2.195	42	97	2.397	73	43	1.21	104	43	1.212
12	2	0.059	43	62	1.699	74	62	1.693	105	14	0.413
13	64	1.758	44	78	2.119	75	13.5	0.393	106	26	0.755
14	61	1.68	45	49	1.357	76	29	0.831	107	45	1.262
15	66	1.839	46	75	2.026	77	76	2.061	108	28	0.805
16	105	2.477	47	98	2.407	78	22	0.641	109	20	0.572
17	106	2.63	48	74	2.003	79	100.5	2.448	110	18	0.518
18	34	0.994	49	73	1.973	80	25	0.732	111	46.5	1.299
19	31	0.893	50	71	1.92	81	37	1.06	112	16	0.473
20	6	0.172	51	81	2.181	82	12	0.56	113	19	0.549
21	53	1.459	52	73	1.981	83	52.5	1.453	114	70	1.898
22	92	2.321	53	66	1.814	84	101	2.513	115	77	2.09
23	33	0.967	54	79	2.133	85	77	2.084	116	65.5	1.788
24	109	2.493	55	115	2.843	86	52	1.43	117	55	1.519
25	51	1.401	56	97	2.394	87	54	1.496	118	74	2.012
26	68	1.861	57	108	2.677	88	67	1.824	119	30.5	0.881
27	79	2.14	58	70	1.895	89	52	1.435	120	50	1.394
28	0	0.056	59	46	1.3	90	83	2.203	121	73	1.98
29	108	2.673	60	65	1.774	91	73	2.033	122	62	1.691
30	71	1.934	61	0	0.051	92	58	1.608	123	59	1.61

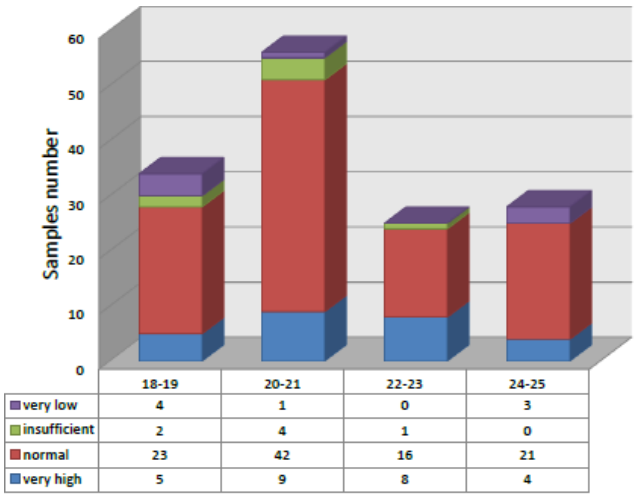
31	51	1.411	62	55	1.526	93	40	1.129	124	49	1.361

Table-1,
continue on next page

Samples Nr.	*Con.*	*A*	*Samples Nr.*	*Con.*	*A*	*Samples Nr.*	*Con.*	*O.D*	*Samples Nr.*	*Con.*	*A*
125	69	1.871	129	67	1.836	133	31	0.943	137	73	1.979
126	74	2.012	130	74	2.001	134	65	1.785	138	25	0.779
127	30.5	0.881	131	29	0.828	135	73	1.983	139	63	1.724
128	80	2.149	132	27.5	0.794	136	67	1.824	140	45	1.319
									141	21	0.593

From epidemiological point of view, it was interesting to demonstrate, if there is a change in number of the unmarried childbearing age females, with adequate protective anti-Rubella IgG antibodies (130/141; 92%), linked to their age, assuming that, as females are going older in age, they would more likely suffer from later natural Rubella virus infection, and subsequently would be seropositive for Rubella IgG antibodies. To answer this hypothesis, obtained results for anti-Rubella virus IgG antibodies in females sera, were further classified according to the age of the assayed females into 4 groups: 18-19, 20-21, 22-23, and 24-25 years old respectively, as shown in Figure-1.

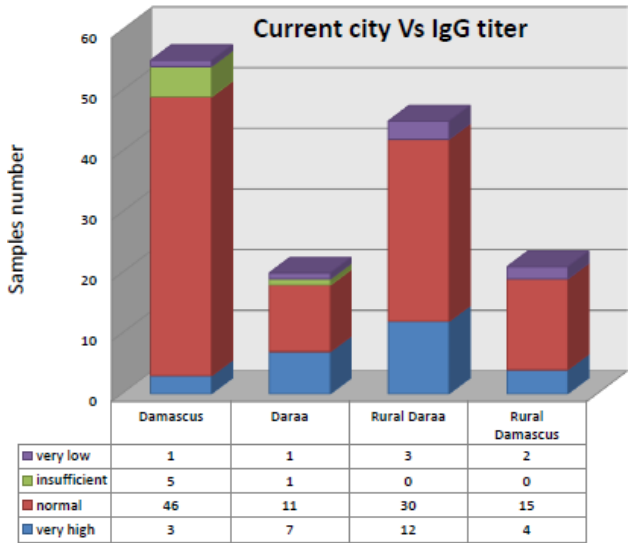
Figure-1: Distribution of the females assayed anti-Rubella IgG antibodies according to their age. Anti- Rubella IgG antibody titers were classified into: very low: less than 12 IU/ml (magenta colored boxes); insufficient: between 12 to 17 IU/ml (green colored boxes); normal: above 17 IU/ml (red colored boxes); and very high: above 100 IU/ml (blue colored boxes) respectively.



The presented data didn't demonstrate a considerable correlation between anti-Rubella IgG titers, and age (coefficient of correlation, r = 0.535), as the number of younger females of 20-21 years old group, with normal, and very high anti-Rubella IgG antibodies titers (42+9/141), was larger than the numbers of older females of 22-23 years old group (16+8/141), and 24-25 years old group (21+4/141) respectively, as shown in figure-1. Furthermore, in an attempt to demonstrate a possible relationship between the site of accommodation and the number of unmarried childbearing age females, who were seronegative for/ or had inadequate anti-Rubella virus IgG antibodies, reflecting the fact that it is possible to link negative anti-Rubella virus IgG antibodies to remote far rural areas, where national MMR vaccine programs could not be reached, or absent. So the obtained sera for anti-Rubella virus IgG antibodies were classified according to the geographic location of accommodation into Damascus city, Daraa city, Rural Damascus, and Rural Daraa, as shown in figure-2. The obtained data didn't demonstrate any correlation between anti-

Rubella IgG titers and the geographic accommodation site (coefficient of correlation, r = 0.098), as the numbers of unmarried childbearing age females with very low, and insufficient anti-Rubella IgG antibodies titers from rural Daraa, and rural Damascus (3+0/141; and 2+0/141 respectively), didn't differ significantly, from the numbers of unmarried childbearing age females with very low, and insufficient anti Rubella IgG antibodies titers from Damascus, and Daraa (1+5/141; and 1+1/141 respectively).

Figure-2: Distribution of the females assayed anti-Rubella IgG antibody titers according to geographic accommodation site of these females in Damascus; Daraa; Rural Daraa; and Rural Damascus. Anti-Rubella IgG antibody titers were classified into very low: less than 12 IU/ml (magenta colored boxes); insufficient: between 12 to 17 IU/ml (green colored boxes); normal: above 17 IU/ml (red colored boxes); and very high: above 100 IU/ml (blue colored boxes) respectively.



Discussion:

In the present study, we assayed sera of 141 unmarried childbearing aged females between 18-25 years old, from Damascus and Darraa governorates, and their related rurals, for anti-Rubella virus IgG titer using a commercial ELISA kit standardized against WHO reference standard serum. Previous published data, defined the existence of protective immunity against Rubella virus infection, when anti-Rubella IgG titer in serum >10 IU/ml[13]. Nevertheless, in the absence of globally one consensus anti-Rubella IgG immunoassay/methodology, this fixed titer cut-off, should be interpreted with caution, as there are many different Rubella IgG immunoassay platforms/kits, already existing in usage, so interpreting laboratory testing results, that correlate with immunity, should accurately reflect the precision, reproducibility, and inter-test system variability in result reporting, even in the presence of an assayed international reference standards[14,15]. Taking these issues in consideration, we have intentionally, classified our obtained anti-Rubella IgG antibodies titers into 4 categories: very low: less than 12 IU/ml, insufficient: (introducing this gray/buffering zone between 12 to 17 IU/ml), normal: above 17 IU/ml, and very high: above 100 IU/ml respectively. We have demonstrated that about 8% (11/141) of these screened unmarried childbearing age females, from Damascus and Darraa governorates are still seronegative for/have not protective anti-Rubella virus IgG antibodies titers in their sera, and subsequently are still susceptible to future Rubella virus infection, possibly at pregnancy. In this study, a clear linkage couldn't be proved between age, or geographic site of accommodation of the studied unmarried childbearing age females, and seroconversion towards protective adequate anti-Rubella IgG antibodies. As females in this study, were selected from high habitants number populated governorates (Damascus, Daraa, and their rurals), which are rather good developed infra-structurally in sanitary, and transport networks, and are well distributed with many primary health care providing centers. So, The continuous presence of Rubella virus infection susceptible unmarried childbearing age females, could reflect that national MMR vaccination programs is not so efficiently implemented to cover all target children population in the different regions of Syria. Actually, it has been observed that, if Rubella children vaccination programs were not optimally implemented, to maintain a herd immunity of 80%

and higher, then a paradoxical increase in congenital Rubella syndrome can occur due to a decreased circulation of the virus and an accumulation of Rubella susceptible adult females[10]. These observations reflect WHO european recommendation of less than 5% susceptibility among women of childbearing age[16].

We compared our results with other epidemiological sero-surveillance studies conducted at neighboring Mediterranean, and surrounding countries: In Northern Greece, 13.9% of women aged between 16-40 years, were susceptible to Rubella[17], In Southern Iran, only 4% pregnant women aged between 16 to 42 years old were susceptible to Rubella[18]. Whereas, In South Italy, 14.2% of pregnant women in 4th to 39th week of pregnancy were susceptible to Rubella[19]. In contrast, in Northern Italy, only 8% of pregnant women were susceptible to Rubella[20]. In Western Turkey, only 3.9 % of pregnant women were susceptible to Rubella[21] , and In Lebanon, 12 % of females[22]. The difference in percentage of Rubella seronegative childbearing age females in our obtained data with those neighboring countries mentioned already may reflect, again, in addition to the different anti-Rubella IgG assay methodologies, the influence of different socioeconomic status, health care systems, and most importantly, vaccination policies, and vaccination coverage efficiency. Similar differences in percentages of Rubella seronegative childbearing age females were reported in other syrian regions[23], and in many european countries[24].

In conclusion, our presented study demonstrated the need for continuous surveillance system for evaluation efficiency of enrolled Rubella vaccination programs in Syria, and the necessity of harmonization of assays used for Rubella immunity determination. Also the importance of introducing a mandatory booster Rubella vaccine at pubescent females. Without actual epidemiological data, Rubella virus infection cases may still occur at higher childbearing age females, with increased incidence of CRS.

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