Detection of Rubella Virus in Aborted Women by IgG Avidity Test

Mohammedsalih Kadir*

Department of Biology, Jimma university, Jimma, Ethiopia

Research Article

ABSTRACT

Rubella virus is the pathogenic agent of the disease rubella. Rubella is predominantly a childhood disease and is endemic throughout the world. Natural infections of rubella occur only in humans and are generally mild. The study aimed to detect Rubella Virus (RV) in aborted women by Immunoglobulin G (IgG) avidity and anti-rubella virus ELISA (IgG) in order to know prevalence of Rubella Virus (RV), to identify at which stage (trimester) it mostly cause abortion in women, and whether it also found in non-aborted women or not.

The study included a collection of forty six samples from women whose age ranges from 18 to 39. Test samples were collected from Omdurman maternity hospital, while control samples were collected from Omdurman teaching Hospital. IgG avidity test was performed using (Euroimmun avidity determination of IgG anti-bodies against RV) kit. The test was performed according to manufactures instruction. Relative Avidity Index (RAI) in percent was calculated by dividing extraction of the sample with urea treatment times one hundred for extraction of the sample without urea treatment. In case of ELISA test by ratio of extinction of control or patient sample to extinction of calibrator. Among the aborted women, 51.35% were in the first trimester, 35.135% in the second, and 13.51% in the third trimesters. 91.89% of test samples show high avidity anti-bodies, while the other 8.11 equivocal range, there is no law avidity. In total sample 93.47% positive high and 6.52% equivocal. In the same way in ELISA test 97.3% is positive, only 2.7% is border line. In total sample 95.65% positive, 2.17% border line, 2.17%. The virus also detected in control sample, but their appearances are relatively lower than it is in serum of aborted women. We can conclude that, rubella virus can be found in aborted women and normal ones, it can cause abortion at high avidity, but it differs in causing abortion from women to women either based on virus's stage, host immunity difference, or other factors. Both IgG avidity index and anti-rubella virus ELISA (IgG) test shows almost the same result. Appearances are relatively lower than it is in serum of aborted women.

Keywords: Rubella virus; Immunoglobulin Gamma (IgG); ELISA; Detection; Relative avidity index

INTRODUCTION

Rubella virus is the pathogenic agent of the disease rubella. It, also known as German measles or three day measles. It is member of Rubi virus genus in family tagoviridae. Rubella diseases, is an infection caused by the rubella virus. Rubella is predominantly a childhood disease and is endemic throughout the world. Natural infections of rubella occur only in humans and are generally mild. Complications of rubella infection, most commonly polyarthralgia in adult women [1]. The disease can affect anyone of any age, but it is rare in infants or those over the age of forty up to 60% of older girls or women experience joint pain or arthritic type symptoms with rubella. The actual burden of Rubella virus is unknown for developing countries, but it is estimated that 110000 Congenital Rubella Syndrome (CRS) cases occur each year world wide [2]. This disease is often mild with half of people not realizing that they are sick. Transmission is typically through inhalation of infectious aerosolized respiratory. Rubella has an average incubation period of 12–18 days, but can extend to 23 days. The infectious period of the virus is from 7 days before to 5–7 days after the onset of rash. A vaccine has been available for several years resulting in significant reduction in new cases according to the latest WHO progress report. However, Rubella has almost been eradicated by immunization programs in many developed countries, but outbreaks amongst the unvaccinated still occur [3]. It is a mild childhood disease that, if acquired during the first 16 weeks of gestation, can result in miscarriage and serious fetal defects. Miscarriage occurs in up to 20% of cases. The severity of the effect of rubella virus on the fetus depends largely on the time of gestation at which infection occurs. Up to 85% infants are infected in first trimester of pregnancy. Some study indicates that; the physician occupational group was more susceptible to rubella. The risk is higher in women of reproductive age. This study aimed to detect anti-IgG virus from the serum sample collected from aborted women in Khartoum state [4].

MATERIALS AND METHODS

Study area
Khartoum state: Test samples were collected from Omdurman Maternity Hospital, control samples collected from Omdurman teaching hospital.
Study duration: From November 2016 to August 2017
Type of study: Case control hospital based study
Study subject: Aborted women of different age group, different, level of education, occupation, tribes, gestational stage, and etc. also unaborted women included as control.
Inclusion criteria: Aborted women with different age group, gestational stage, tribe, blood group, Occupation, and educational level.
Exclusion criteria: Male, girls with age below 18 and women above 39.
Ethical consideration: Permission from hospital was applied and verbal consents were taken from the women involved in this study.
Sample size: Sample size n-46 (37 test sample and 9 control sample)
Collection of blood samples: Blood samples were collected using 5 ml syringe into EDTA (Ethylenediaminetetraacetic acid) container.
Sample processing and quality control: Each blood sample was centrifuged at 3000 g for 5 minutes, and then plasma was gently collected into plain container and stored at -20°C until the serological analysis [5-10].

Procedure
IgG avidity test was performed using (Euroimmun avidity determination of IgG anti-bodies against Rubella viruses) kit. The test was performed according to manufactures instruction as follow:
Sample incubation: 100 μl of each controls or diluted patient samples was transferred into individual microplate wells, then Incubated for 30 minutes at room temperature. Washed one time using 300 μl of working strength wash buffer.
Urea incubation: 200 μl urea solution was added into the first wells (odd column 1,3,5 etc.) of micro titers strip and 200 μl of phosphate buffer in parallel column (even column 2,4,6, etc.) of micro titer. Incubated for 10 minutes at room temperature. Then washed three times using working strength wash buffer for each wash [12].
Conjugate incubation: 100 μl of enzyme conjugate (peroxide labeled anti-human-IgG) was added in to each microplate wells. Then incubated for 30 minutes at room temperature. Then washed three times using working strength wash buffer for each wash [13].
Substrate incubation: 100 μl of chromogen/substrate solution was added in to each of the microplate wells. Then incubated for 15 minutes at room temperature.
100 μl of stop solution was added into each of the micro wells in order to stop reaction. Slight shake of microplate was done before measuring in order to ensure a homogeneous distribution of the solution.
Photometric measurement of colour intensity was made at a wave length of 450 nm and a reference wavelength

between 620 nm and 650 nm.

Result calculation: The Relative Avidity Index (RAI) for each sample and control was calculated by dividing extraction of the sample with urea treatment times one hundred over extinction of the sample without urea treatment.

$$RAI\ in\% = \frac{extinction\ of\ sample\ treated\ with\ urea * 100}{Extinction\ of\ sample\ without\ urea}$$

Result interpretation

The results were interpreted according to manufacture guides as follow: RAI<40%: indicate law avidity antibodies.

RAI 40%-60%: Indicate equivocal range. RAI>60%: indicate high avidity antibodies [14].

The mechanism of ELISA (IgG) is similar to that of avidity. The only difference is that, there is no urea incubation [15-18]. The result was evaluated semi-quantitatively by calculating the ratio of the extraction of the control or patient sample over the extraction value of calibrator 3.

=extinction of the control or patient sample

-- extinction of calibrator 3

The results were interpreted according to manufacture guides as follow:

Ratio<0.8: Negative

Ratio ≥ 0.8 to <1.1: Borderline ratio ≥ 1.1: Positive.

RESULTS

Among the aborted women, 19/27 (51.35%) were in the first trimester, 13/37 (35.135%) in the second, and 5/37 (13.51%) in the third trimesters. 34/37 (91.89%) of test samples show high avidity anti-bodies, while the other 3/37 (8.11%) equivocal range, there is no law avidity (Figures 1 and 2). In total sample 43/46 (93.47%) positive high and 3/46 (6.52%) equivocal (Table 1). In the same way in ELISA test 36/37 (97.3%) is positive, only 1/37 (2.7%) is border line (Figures 3 and 4). In control samples 8/9 (88.8%) was positive, and 1/9 (11.2%) was negative; there is no border line. In total sample 44/46 (95.65%) positive, 1/46 (2.17%) border line, 1/46(2.17%) (Table 2 and Figure 5).

Even though anti-virus IgG is also detected in control sample, but their appearances are relatively lower than it is in serum of aborted women (average 83.3 in test sample, while it is 73.12 in control samples) [19].

Figure 1. Frequency of RV IgG avidity according to gestational stage.
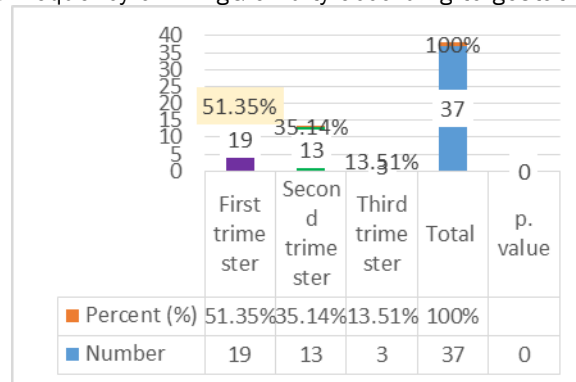
P. Value=0.042

There is statically significant association between gestational stage and avidity result at (P=.05)

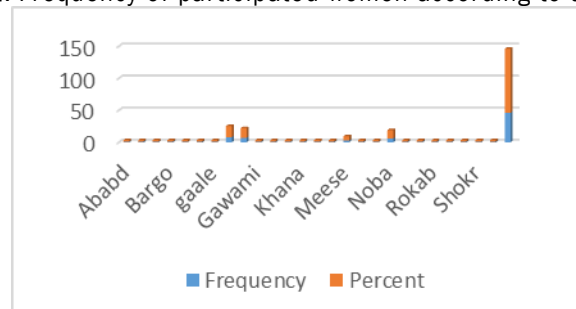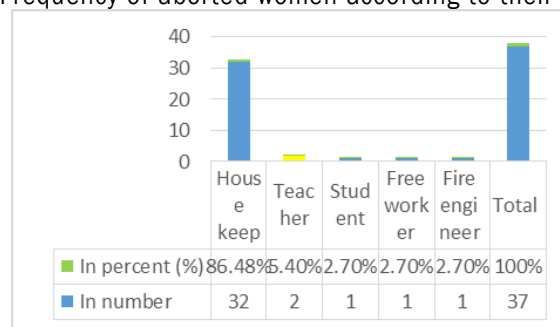**Figure 2.** Frequency of participated women according to their tribe.

Table 1. Frequency of anti-rubella ELISA test.

| Results	Case		Control		Total	
	In no.	Percent (%)	In no.	Percent (%)	In no.	Percent (%)
Negative	0	0	1	11.2	1	2.17
Border line	1	2.7	0	0	1	2.17
Positive	36	97.3	8	88.8	44	95.65
Total	37	100	9	100	46	100

Figure 3. Frequency of aborted women according to their occupation.

P. V=0.014
There is statically significant association between occupation and RV IgG avidity result at (P=.05)

Figure 4. Frequency of aborted women according to their educational level.
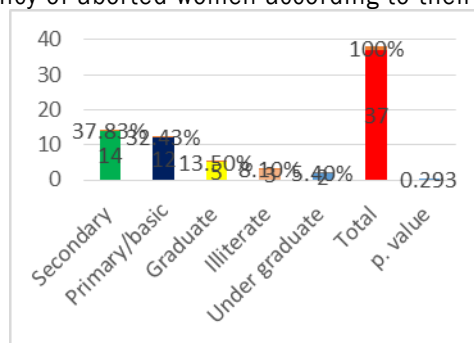

P. value=0.014
There is statically significant association between educational level and avidity result at (P=.05)

Table 2. Relationship of anti-rubella ELISA IgG with different risk factors.

Anti-rubella ELISA (IgG) result with gestational stage		Anti-rubella ELISA (IgG) result with age group		Anti-rubella ELISA (IgG) result with occupation		Anti-rubella ELISA (IgG) result with level of education	
Gestational stage	PAH/RAI in (%)	Age group	PAH/RAI in (%)	occupation	PAH/RAI in (%)	Educational level	PAH/RAI in (%)
Frist trimester	51.32	18-28	70.27	House keep	86.48	Secondary	37.83
Second trimester	35.14	29-39	29.79	Teacher	5.4	Primary	32.43
Third trimester	13.51			Student	2.7	Graduate	13.5
Total	100	Total	100	Fire engineer	2.7	Illiterate	8.1

P. value	0.22	P. value	0.4	Free worker	2.7	Under graduate	5.4
				p. value	0.4	p. value	0.11

Figure 5. Frequency of blood group for test sample.

	A	B	O	Total
Percent	21.61	5.4	72.97	100
Frequency	8	2	27	37
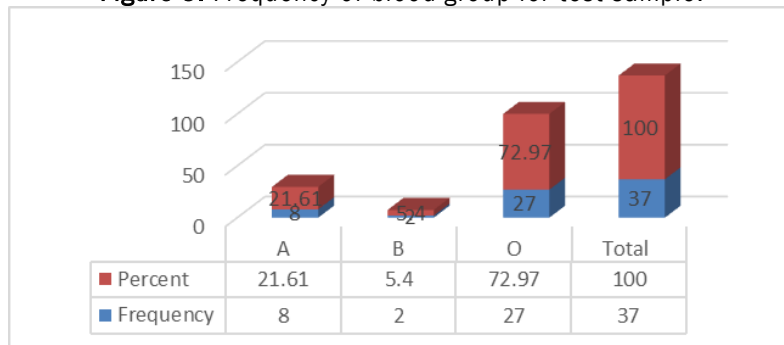
DISCUSSION

The high rate of morbidity and mortality caused by rubella in many countries; especially to fetus of infected mothers has made the screening of aborted and pregnant women an important research activity and very important area of study. This study specifically emphasized on the aborted women. Among the thirty seven test samples 3/37 (8.11%) shows equivocal range, while the remaining 34/37 (91.89%) high avidity anti-bodies, there is no law avidity as indicated above. This indicates that rubella cause abortion mostly at high avidity. It also indicates that most of women (93.47%) participated in this study have re-infection or past infection. High avidity usually associated with re infection or past infection, while law avidity is associated with recent primary rubella infection. Result of ELISA IgG showed that, 36/37 (97.3%) of the test sample was positive. This study finding was almost similar to that observed in 2012 in Khartoum state. It showed that 38/39 with history of abortion (97.43%) were positive for IgG RV while the rest 1 (2.6%) were negative [20].

Information from aborted women indicates: 19/37 (51.35%) were in the first trimester, 13/37 (35.135%) in the second and 5/37 (13.51%) in the third trimesters. This indicate its impact is highest at early stage of pregnancy. This justifies the idea that if rubella acquired during the first 16 weeks of gestation, can result in miscarriage and serious fetal defects.

29/37 (78.37%) had history of pregnant before while the remaining 8/37 (21.62%) have no history of pregnant before; 16/29 (55.17%) of them had history of abortion before; 15/16 (93.75%) of them had two times, while remaining 1/16 (6.25%) was four times. Regarding their occupation 32/37 (86. 48%) of them house keeper, 2/37 (5.4%) teacher, 1/37 (2.7%) student, 1/37 (2.7%) fire engineer, and 1/37 (2.7%) free worker (Figure 2 above). based on this study house keeper are most affected professional group; This may be because of law prevention management of inhalation and skin contact among the family member. In contrast to this, study done in Japan in April 2013 indicates that, the physician occupational group was more susceptible to rubella. Study not includes only aborted women. Regarding their level of education 14/37 (37.83%) secondary, 12/37 (32.43%) primary, 5/37 (13.5%) graduate, 3/37 (8.1%) illiterate, and 2/37 (5.4%) under graduate. Despite the majority of them being educated, they are at law level of education, and with a low level of awareness of rubella infection. Among participating tribes Gaale 9/46 (19.6%), 7/46 (15.2%) Gamoa, 6/46 (13%) Noba, 4/46 (8.7%) Meese and each of the remaining tribes share 1/46 (2.2%). Even though this study indicate that Gaale is the largest number affected, Gamoa is the second and Noba is third we cannot conclude that they are most susceptible tribe to RV until we compare their percent among Khartoum's population and until study done with large number of size. Regarding blood group 27/37 (72.97%) of them O blood group, 8/37 (21.61%) A and 2/37 (5.4%) are B. based on this study woman with O blood group are highest in number. This may be O blood group is at high risk factor of RV or because O blood group is the most common group. Age group 70.27% range from 18-28, while 29.72% ranges from 29-39. This study finding was similar to those observed in 2012 in Zimbabwe. It showed that 60% of affected women in the age range 21-25, and also with those observed during the pre-vaccine era in other regions. This is high reproductive age.

Some studies have showed that as age increase a woman has a better chance of attaining Rubella immunity naturally without getting vaccination.

CONCLUSION

We can conclude that, RV can infect all women: aborted and un aborted, it can cause abortion at high avidity when it is past or re infection, but it differs in causing abortion from women to women either based on virus's stage, host

immune system, or other factors. Both IgG avidity index and anti-rubella virus ELISA (IgG) test shows almost the same result. Its impact is highest at early stage of pregnancy (first trimester). Also we can say Khartoum state is endemic area for rubella.

Recommendation

It is advisable for girls to take vaccination pre-marriage; as much as possible pregnant women should avoid contact with people that have skin rash; national health institutes should give emphasize for this issue, introduce vaccine, promote awareness for women of reproductive age, particularly pregnant women. As I recommend researchers to work more to identify factors behind why it cause abortion in some women not in others, whether it different in rural and urban based on different factors like air pollution, occupation and others with increasing sample size.

REFERENCES

1. Ackerknecht EH, et al. A short history of medicine. Baltimore: Johns Hopkins University Press. New York. United States of America. 1982;129.
2. Agbede OO, et al. Sero-Prevalence of Antenatal Rubella in UITH. Open Public Health J. 2011;4:10–16.
3. Alton ME, et al. Prenatal diagnosis. N Engl J Med. 1993;328:114–120.
4. Assaad F, et al. Rubella world impact. Rev Infect Dis. 1985;7:29–36.
5. Atkinson W, et al. Epidemiology and prevention of vaccine preventable diseases. 10th Edition. CDC Stacks Public Health Publications. USA. 2007.
6. Atreya CD, et al. Rubella and birth defects: Molecular insights in to the viral teratogenesis at the cellular level. Birth Defects Res A Clin Mol Teratol. 2004;70:431-437.
7. Banatvala JE, et al. Rubella. Lancet. 2004;363:1127–1137.
8. Bardeletti G, et al. "Morphology, biochemical analysis and neuraminidase activity of rubella virus". Arch Virol. 1975;49:175–186.
9. Beatch MD, et al. "Interactions between rubella virus capsid and host protein p32 are important for viral replication". Virol J. 2005;79:10807.
10. Bosma TJ, et al. "PCR for detection of rubella virus RNA in clinical samples". J Clin Microbiol. 1995;33:1075-1079.
11. Brooks GF, et al. Medical microbiology. 25th Edition. McGraw-Hill Companies. New York. USA. 2010.
12. Bullens D, et al. Congenital rubella syndrome after maternal reinfection. Clin Pediatr (Phila). 2000;39:113–116.
13. Chen MH, et al. Mutagenic analysis of the 3 cis acting elements of the rubella virus genome. J virol 1999;73:3386–3403.
14. Coulter C, et al. Rubella infection in pregnancy. Commun Dis Intell. 1999;23:93-96.
15. Curdt I, et al. "Development of fully automated determination of marker specific immunoglobulin G (IgG) avidity based on the avidity competition assay format: Application for Abbott Architect cytomegalovirus and Toxo IgG Avidity assays". J Clin Microbiol. 2009;47:603–613.
16. Dominguez G, et al. Sequence of the genome RNA of rubella virus: Evidence for genetic rearrangement during Toga Virus Evolution. Virology. 1990;177:225–238.
17. EL-Mekki AA, et al. Screening of rubella antibodies among Saudi women of child bearing age. Saudi Med J. 1998;19:575-577.
18. Farquharson RG, et al. "Updated and revised nomenclature for description of early pregnancy events." Hum Reprod. 2005;20:3008–3011.
19. Forrest JM, et al. Gregg's congenital rubella patients 60 years later. Med J. 2000;177:664–667.
20. Frey TK, et al. Molecular analysis of rubella virus epidemiology across three continents, North America, Europe, and Asia, 1961–1977. J Infect Dis. 1998;178:642-650.