Gene Sequence of Rubella Virus Infection in Serum of Wives That Correlate to Intrauterine Insemination Success Rate in Baghdad's Population Couples

Loqman Juma. Tawfiq
Medical Technical Institute/Baghdad, /Middle Technical University /Iraq (MTU).Technical Nursing Dept. First Aid branch.

ABSTRACT
The current study focused on the prevalence of Rubella virus disease among wives who underwent the process of artificial insemination (IUI) and its relationship to the elevated proportion of free radicals in the blood plasma and its correlation with water pollution in the spreading of the disease for the period from 15/2/2022 to 1/2/2023 in private laboratory for IUI procedure (AL-Amal). The study included 60 women who underwent (IUI) procedure and have positive results for Rubella virus (IgG and IgM) compared to wives who tested negative for Rubella virus for ages groups from 20 to 35 years.

The study focused on the impact of the above-mentioned diseases on the success rate of the operation among the group of infected and uninfected women, based on the examination of Rubella virus IgG and IgM testes. The results indicated that there are high statistic levels in terms of infected and uninfected women (p <0.01). By using the RT-PCR test, only two out of sixty specimens containing pregnant women have a rubella viral RNA positive proportion of 3.33%. The seropositivity of rubella antibodies was shown to be significantly correlated (P<0.05) with the history of abortion, with 61.42 % of pregnant women having experienced one. As a result of many IUI stage, potential roles of oxidative stress (as concentration of Malondialdehyde in human serum) have been implicated. In this prospective cohort study, due to female factor infertility, there was a highly significant deference between low and high value of Malondialdehyde an oxidative stress marker (P<0.001).
Aims: To find the relationship of oxidative stress and Rubella virus infection with percent of successful IUI by using Rubella virus IgG and IgM antibodies and also by using PCR method for gene selection for the virus.

KEYWORDS: IUI; water Pollution ; Rubella virus; Pollution: RT-PCR test.

INTRODUCTION
In the world, there are now seven cases of rubella per 1,000,000 persons, down from fifteen in 2010 (1). Rubella Laboratory Network and Global Measles witch belong to WHO provided standardized, quality-controlled laboratory investigation for 193 of the 194 countries where rubella surveillance was carried out in 2020. In 2012, there were reports of rubella cases from 175 nations; by 2019, this number had risen to 179. However, during the COVID-19 pandemic in 2020, this number dropped to 135 (2). Rubella seroprevalence was determined by ELISA tests (quantitative and qualitative IgG and IgM) in which 90.2% of participants
were IgG positive for rubella and 0% were IgM positive (3). Rubella virus (Rub.V) is a positive-sense RNA virus and as a Rubivirus genus member and Matonavidae in the family (4). Rubella may prevented by special vaccine for it by this rout, the distribution of the disease will be minimized and the prevent signs and symptoms, viral illness witch it can spread by the respiratory system and the obvious symptoms of the disease in the infected person include: low-grade fever, sore of the throat, lymphadenopathy (5). Besides acute viral infection occurs in children and can be very dangerous for a pregnant woman, in witch can result in miscarriage, and have extremely effect the developing fetus. If the infection occurs during the first trimester congenital defects and death (6). Important information and data were collected in India from the period between 2016 and 2018, the deformities that affect the fetus of a mother infected with the virus can be summarized as follows (structural heart defects 78.8%, and had one or more eye signs (cataract and glaucoma) 59.9%, 38.6% had hearing impairment and Congenital Rubella Syndrome (CRS) by 24.1% (7). CRS is very high if the fetus who have rubella infection between 12 to 20 weeks and after 20 weeks of pregnancy respectively (8). For the diagnosis of Rubella and congenital Rubella detection is performed by serological methods of rubella-specific IgM of both postnatally, congenitally acquired rubella and newborn infant. Isolation the RNA of the virus by (RT-nPCR) witch mean (nested reverse transcription-polymerase chain reaction) that is one of the most sensitive methods for diagnosing the presence of the virus in congenital rubella in the fetus and newborn (9).

Intrauterine insemination is an assistant reproductive technology (10). There is a general agreement on the preference of intrauterine insemination to the more expensive and invasive assisted reproduction methods and offering it as the first choice treatment in moderate and unexplained male and female factors subfertility cases (11).

The justification of intrauterine insemination IUI with the sperm of the husband, is that it leads to bypass of the cervical–mucus barrier and increases the motile sperm numbers with highly normal form proportions at the fertilization site (12). In the few past decades, homologous artificial inseminations were only carried out in male subfertility and psychological dysfunction cases, like retrograde ejaculations, impotence and hypospadias. With the routinely application of post-coital examination, other indications method will be important like immunologic causes and hostile cervical mucous (10). Indeed, the interest in IUI is related to the refinement of methods for washed motile sperm preparation. Such washing methods are required for removing infectious agent, prostaglandins, antigenic protein, non-motile sperms, WBCs and immature germ cell (13). This can improve spermatozoa quality via reducing free oxygen radical formation following spermatozoa preparations. Finally, it will lead to improvement of invivo and invitro fertilizing capability of the sperms (11). In the majority of moderate and unexplained male subfertility cases, published data indicated that treatment initiation with IUI was shown to be more cost effective than IVF (10). Surprisingly and regardless of evidenced argument, data from New Zealand and Australia noticeably showed that about 80% of fertility facilities are persuaded that IUI is cost-effective, however, about 33% of these facilities still uphold IVF as the first line treatment even with patent tubes and normal seminal properties (13). Such procedure can be really interesting for resource-poor countries, because IUI programs are easily performed. These methods and techniques are easily learnt and low costs in comparison with IVF/ICSI as well as very rare serious complications (14).
is an. The imbalance between reactive oxygen species (ROS) and antioxidant defense is defined as oxidative stress (OR) (4). The most important molecules determined in human serum are Malondialdehyde, which are produced by lipid peroxidation cascade process that ends with low-molecular electrophilic aldehyde production e.g. Malondialdehyde (MDA) and 4-hydroxynonenal. Remarkably, MDA is able to bind with mitochondrial proteins resulting electron leakages and formation of cellular reactive oxygen species (ROS), leading to worsened oxidative stresses (15). Antioxidant enzymes such as copper–zinc containing superoxide dismutases (Cu, Zn-SODs), manganese-containing SODs (Mn-SODs), glutathione peroxidase (GPX) and catalases are the systems of body defense against oxygen-species oxidizing action (16).

In spite of substantial progresses in the assisted reproductive technology (ART), there is a remarkable low rate of success in this method. Several factors are shown to affect the outcomes of IUI and ART (17). Oxidative stress is regarded as one of the most essential factors which influence different IUI and ART procedure steps and its unavoidable outcomes (4).

ROS is increased as a result of some environmental conditions and diseases such as obesity, smoking and nutrition, and can cause many pathologic disorders like cancers, periodontitis, neurodegenerations, cardiovascular disease, diabetes and renal disease (18). It was demonstrated by previous studies that endometrial secretions and menstrual discharges are novel samples to identify oxidative stress biomarker (19).

During IUI and ART procedures, implantation and early post implantation developmental periods are important steps, and the outcomes of a complete ART process largely depends upon their successes. In previous studies, it was suggested that during this stage, there is a highly sensitivity of developing organisms to ROS-induced oxidative damages. Any probable damage may result in a negative effect on pregnancy establishment (20). Idiopathic recurrent pregnancy losses and spontaneous abortions are the contrary effects of oxidative stress on pregnancy (21). Successful implantations require appropriate interactions between the embryos and endometrium. It was revealed by previous studies that antioxidant enzymatic systems were involved in the embryo’s development as well as their receptive uterine endometrium prior to implantations (22). There are many methods to assess endometrial receptivity and maturity (23). In one of the methods, endometrial secretions are analyzed during implantation windows. It was demonstrated by recent studies that endometrial secretions may denote the interactions between an embryo and an intrauterine environment (24),(25). In addition, it was shown that aspirations of endometrial secretions before embryo transfers may be carried out without influencing the implantation rate (26) (27). The role of cytokines including IL-1β or TNF-α in anticipating outcomes of in vitro fertilization (IVF) was investigated. To the best of our knowledge, there was no previous analysis of endometrial secretion to identify the oxidative stress markers.

In the current study, our aim was to investigate whether there was an impact of oxidative stress markers on IUI & IVF success.

MATERIAL AND METHOD:
Blood serum was taken from arm blood vessel of women (60 which have positive for toxoplasmosis and 30 with negative results as control cases) by disposable 5mm syringe.

With using Lab. Centrifuge we separate the serum to be used finally for laboratory assay.

Kit stripe (Rubella virus IgG and IgM) were used to determined positive and negative results.

The current case-control seroprevalence study was designed to detect anti-Toxoplasma IgG antibody and anti-Rubella IgM antibody levels using the enzyme-linked immunoassays.

RTPCR tests are also employed to molecularly detect viral RNA associated with infectious rubella (Saccace, Italy supplied the RNA extraction reagent and real-time PCR search for qualitative identification of infectious rubella).

For MDA assaying, the Bioxytech MDA-586 spectrophotometric The analysis was conducted using a reagent (Catalogue No: 21044) obtained from Oxis International, Inc. (Foster City, CA, USA) in strict adherence to the manufacturer's instructions.

The keyword, target, catalog number or product were searched.

IUI was done by simple layer and centrifuged method. After 20-30 minute the supernatant which contain the active sperm and the solution of activation buffer was obtained using a syringe.

The syringe was linked (which contain the seminal fluid sample of husbands) to a special IUI catheter.

By Gynecologist, the sample was housed in to the wife uterus.

After one hour, the wife was allowed to go home.

Pregnancy test was done after 10 days to IUI.

Statistical analysis: Data were analyzed by the SPSS Vr.24 program, and the t-test with Mont Carlo test (MCP) were analyzed at 5% &1% level of significances.

RESULTS:

Table (1) showed the distribution of age groups of 60 wives which explain the role of age in successful IUI procedure. There was an adverse effect of age on this technique.

Table (1): Numbers of wives and their age groups

<table>
<thead>
<tr>
<th>Numbers</th>
<th>Age</th>
<th>Percent of IUI succeeded</th>
<th>P Value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1: 15</td>
<td>20 - 25</td>
<td>23%</td>
<td>G1 and G2 P&lt; 0.01</td>
<td>Yes</td>
</tr>
<tr>
<td>G2: 20</td>
<td>26- 30</td>
<td>17%</td>
<td>G2 and G3 P&lt; 0.01</td>
<td>Yes</td>
</tr>
<tr>
<td>G3: 25</td>
<td>31 - 35</td>
<td>13%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2- Table 2 indicates the number of wives that have positive and negative IgG or/and IgM of Rubella virus.

Table (2): Results of IgG and IgM in wives with Rubella virus.

<table>
<thead>
<tr>
<th>Numbers of wives</th>
<th>IgG</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>4</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>2</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>30</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>

3- The findings indicated that there was a statistically significant distinction in the presence of IgG+ or IgG- and IgM+ or IgM+ in spouses who underwent successful IUI (Table 3).

Table (3): Number of IUI succeeded and presence of anti Rubella virus (Rub. Virus) IgG and IgM.

<table>
<thead>
<tr>
<th>No. of IUI succeeded</th>
<th>Rub. virus IgG positive</th>
<th>Rub. virus IgM positive</th>
<th>Rub. virus IgG negative</th>
<th>Rub. virus IgM negative</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>9 (60)</td>
<td>2 / 60 (%)</td>
<td>4 / 60 (%)</td>
<td>1 / 60 (%)</td>
<td>3 / 60 (%)</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>4 (60)</td>
<td></td>
<td></td>
<td>1 / 60 (0.6 %)</td>
<td>3 / 60 (1.8 %)</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>1 (60)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P &lt; 0.05</td>
</tr>
</tbody>
</table>

4- The findings revealed a substantial and statistically significant disparity in the serum MDA concentration between spouses who were undergoing IUI and those who were infected with the Rubella virus. Table (4).

Table (4): MDA concentrations in serum of wives infected with Rubella virus and control groups enrolled in IUI procedure.

<table>
<thead>
<tr>
<th>Age</th>
<th>Patients MDA µM mL⁻¹</th>
<th>Controls MDA µM mL⁻¹</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 - 25</td>
<td>335±38.16</td>
<td>295.66±40.32</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>26-30</td>
<td>342±43.52</td>
<td>312±42.23</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>31 - 35</td>
<td>351±44.78</td>
<td>321±45.43</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>
5- The results find that there are a statistically significant difference in wives succeeded by IUI procedure in relation to presence of IgG positive and negative results (P < 0.05) Fig.(3).

![Graph showing comparison between successful IUI with IgG positive or negative](image)

**Fig (3):** Comparison between successful IUI with IgG positive or negative.

6- The findings indicated that there was a statistically significant distinction among spouses who underwent IUI regarding the occurrence of positive or negative IgM results (P < 0.01). Fig.(4).

![Graph showing number of IUI succeeded in relation to presence of Rubella virus. IgM positive or negative](image)

**Fig (4):** Number of IUI succeeded in relation to presence of Rubella virus. IgM positive or negative.
Discussion

It was shown that Rubella virus is a crucial disease owing to its worldwide distribution and variable kinds of diseases it causes. The virus has been known to cause certain critical impacts on the reproductive capability for men and women (28).

Thus, the purpose of the present case-control investigation was to determine the rate of rubella virus seropositivity among subfertile females who underwent IUI. This result approximates other study who estimated that it have negative role for the virus on human general and reproductive capacity (29). This high prevalence is attributable to a lack of health education and airborne exposure to risk factors, such as the respiratory secretions of infected individuals (29).

The results of regional prevalence of Rubella virus seropositivity was higher than those of other studies such as studies conducted in Rabat, Morocco 85% (30), Western Yemen for IgG antibody is 89.2% (31) and in Italy 72% (32). Nevertheless, these findings were less than those estimates of 89% among blood donor individuals in the southern Ethiopia (33).

Our results reported a highly significant prevalence (p<0.01) of the virus in subfertile females (54.75%) when compared to the controls (35.66%), and this may suggest a positive relationship between Rubella and infertility. The results in our study agreed with the finding of (29) who detected a higher prevalence (15.9%) of Rubella IgG antibodies by ELISA in infertile females when compared with pregnant women 5.6%. A positive correlation between Rubella infection and infertility is mediated by the development of endometritis and foetal rejections, which result from the virus being released from dormant structures in endometrial tissues in response to stimulations during placental formation. (34).

In the current study, the prevalence of Rubella virus IgG antibodies among the older age group (30-39) years was found to be higher than other age groups 52.65% in infertile females and 33.7% in pregnant women. This finding was consistent with other studies regarding increasing Rubella virus seropositivity with age (35).

The results of our study indicated that the indicators of oxidative stress (OS) in the endometrial secretion may be representing the uterine receptivity status before the embryonic transfer. A positive correlation has been observed between elevated levels of antioxidants (SOD, CAT, or TAP) and decreased levels of oxidative stress indicators (LPO) and favorable institutional conceived outcomes (IVF). Formerly, the association between female fertility and oxidative stress was inspected in many studies (36), (37). An imbalance between the body's antioxidant defiance and the overproduction of reactive oxygen species (ROS) induces oxidative stress, which is triggered by advancing age and diseases that impact female reproduction. Oxidative stress is induced by various conditions, including endothelial dysfunctions, recurrent or premature infant loss, hypertension, premature births, ectopic pregnancy, and gestational diabetes. Bad lifestyle habits and environmental pollutant can lead to a worsened oxidative stress, suggesting a possible role for antioxidants to mitigate such impacts and improve fertility (38).

CONCLUSIONS
It can be concluded from our study that there was a high Rubella IgG antibody seropositivity in females in Baghdad population. Accordingly, health education programs are necessary to primary Rubella prevention. In addition, infertile females showed a higher significant prevalence of the virus than the control group particularly among older age groups. The results of this study suggest a potential correlation between the Rubella virus and infertility; therefore, additional research is advised to elucidate the underlying mechanism of this association.

REFERENCES:


2833-7433 /© 2024 The Authors. Published by Academic Journal INC. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)


