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Relationship between Rubella Virus in Women and anti-dsDNA levels

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Abstract:

Using a cross-sectional methodology, blood samples were subjected to ELISA analysis to quantify rubella IgM, rubella IgG, and anti-dsDNA antibody concentrations in order to ascertain the correlation between rubella immunological indicators, specifically IgM and IgG, and anti-dsDNA antibody concentrations in a sample of fifty-nine reproductive-age women; Rubella IgM levels were consistently low throughout the study group, indicating the absence of acute infection, while rubella IgG levels showed significant variability, likely reflecting differences in prior exposure or immunity. Anti-dsDNA antibody levels were highly variable; some showed markedly elevated concentrations, indicating subclinical autoimmune activity; a noteworthy finding was the progressive increase in anti-dsDNA levels among women who had two or three abortions, a group that also showed the most prominent outlier titers, strongly indicating the involvement of autoimmune processes in recurrent pregnancy loss within a particular subset of women; participants who had six or more abortions showed normal dsDNA levels, indicating that extreme recurrent pregnancy loss is likely due to non-autoimmune causes, Rubella IgM levels did not correlate with anti-dsDNA, but a concentration of elevated dsDNA values in women with moderately elevated IgG levels suggested possible immune-priming effects from previous rubella exposure. These findings support the idea that autoimmune activity, rather than an active viral infection, may contribute to pregnancy loss, highlighting the need to incorporate autoimmune screening, including anti-dsDNA testing, into the clinical assessment of unexplained or recurrent miscarriage. Additionally, the study highlights the importance of rubella immunity surveillance and suggests future research on viral–autoimmune interactions in the context of reproductive health.

Keywords: Age, dsDNA, Women, Rubella, Infection

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Introduction:

Rubella is an enveloped, single-stranded RNA virus from the genus Rubella virus, which constitutes a critical public health threat in women of reproductive age, given that the first infection during early pregnancy is linked to miscarriage, fetal death, or congenital rubella syndrome (CRS) [1]. Worldwide immunization action efforts have led to massive reductions in CRS; nevertheless, serology research routinely shows very high rates of rubella-susceptible women, generally varying between 5% and 15% in those of reproductive age, especially in low- and middle-income areas [2]. The recent studies in North Africa, India, and the Middle East show that the seroprevalence of rubella IgG for women of reproductive age is estimated between 85% and 96%, leaving a considerable population at risk of primary infection in pregnancy [3; 4]. This immune deficiency has clinical importance when screening of seronegative women pre-conception and systematic vaccination of women is not routinely applied to all. Apart from its teratogenic effects, rubella is associated either with the initiation of or the modification of autoimmune responses [5]. Several lines of evidence implicate rubella infection or vaccination to induce or amplify autoantibody generation through mechanisms of molecular mimicry, bystander activation, and epitope dissemination [6].

Autoimmune consequences were described in details individually with rubella infection or immunization; such as islet-mediated autoimmunity and type 1 diabetes; autoimmune thyroid disease, and the higher incidence of anti-rubella IgM in a wide range of autoimmune disorders [7]. In Egypt recently study was conducted, individuals with systemic lupus erythematosus exhibited significantly elevated anti-rubella IgG titers in comparison with controls, with rubella IgG titers correlating with disease-associated biochemical indexes suggesting that rubella-specific humoral mechanisms also serve as an indicator for SLE activity [8]. The study suggest that rubella exposure may contribute to the development of systemic autoimmunity; initiation or maintenance in genetically susceptible individuals [9]. Anti-double-stranded DNA (anti-dsDNA) antibodies, well-established serological markers for systemic lupus erythematosus for precise specificity, are required for the pathogenesis of SLE [10]. They are closely related to immune-complex deposition, complement stimulation and tissue damage, especially in lupus nephritis and often show matching titers with disease activity [11].

Moreover, the common infection caused by SLE disproportionately affects women, with the highest rate observed during reproductive years and a female-to-male ratio as high as 9:1. Thus, anti-dsDNA positivity is especially important for this population [12]. Importantly, anti-dsDNA and other autoantibodies can be detected years prior to SLE presentation, indicating a considerable pre-clinical period of subclinical autoimmunity [13]. In women of reproductive age, anti-dsDNA positivity is associated with adverse reproductive outcomes, including miscarriage, preterm birth, and lupus exacerbations, while in pregnancy, in addition to impaired success of IVF in ANA–positive infertile women [14].

There is likely a presence of rubella virus (RV), which could remain in the cells and has shown associations with autoantibodies in the endocrine and rheumatic diseases, and in the population most affected by SLE and anti-dsDNA conditions [15]. The exact relationship between rubella exposure and anti-dsDNA positive status in otherwise healthy female patients of reproductive age is not yet well understood. Existing sero-epidemiological studies have largely focused either on rubella immunity and congenital rubella syndrome prevention or on

autoantibody profiles among established systemic lupus erythematosus, with very few studies investigating rubella-specific antibodies in concert with lupus-associated autoantibodies [16].

Research investigating whether natural or vaccine-induced protection against rubella is associated with an increased prevalence or titering of anti-dsDNA antibodies in this population may provide important information about viral effects on pre-clinical autoimmunity, help identify women at an elevated risk for subsequent systemic lupus erythematosus or adverse pregnancy outcomes, and improve counseling about vaccination and reproductive planning. This research aims to evaluate the relationship between rubella infection/immunity and anti-dsDNA positivity in women of reproductive age, in combination with virological and immunological markers, to explain the potential role of rubella-related immune responses in the early stages of systemic autoimmunity.

Materials and methods:

The work of the present investigation was cross-sectional analytical to study the association between rubella serological markers IgM and IgG in line with anti-dsDNA antibody positivity of women of childbearing age. The research was done in Erbil, Kurdistan Region of Iraq, from January to June 2025. The collected sample was analyzed in laboratories at the Department of Medical Laboratory Technology. Standard quality control protocols of biosafety and safety regulations were observed in these facilities. This design was chosen to enable concurrent measurement of viral immunity markers and autoimmune antibodies, which would enable assessment of possible associations with reproductive trajectories, including abortion history. A sample of 59 women aged 13–44 years was recruited through convenience sampling from outpatient clinics, health care facilities with family medicine centers, and visiting laboratories for routine serology exams. Eligibility criteria were: (1) the female reproductive age, (2) consent of the respondents, and (3) availability of all clinical and demographic information. Exclusion criteria included: (1) current pregnancy, (2) previously diagnosed autoimmune disease, (3) continued immunosuppressive therapy, and (4) current acute infection, either clinically or from laboratory investigations. Participants were then interviewed by an open questionnaire, which included demographic information, blood group, and obstetric history, including previous abortions.

Ethical approval was obtained from the institutional review committee, and written informed consent was obtained from all participating individuals and minor guardians. Approximately 5 mL of venous blood in sterile disposable vacutainer systems was taken from each participant. Blood samples were taken in a plain (non-anticoagulant) tube, allowed to clot at room temperature for 20–30 min, and then centrifuged at 3000 rpm for 10 min to obtain clear serum. Serum aliquots were then immediately transferred into sterile Eppendorf tubes and kept at –20°C until the analysis.

All samples were processed within 48 hours of collection to ensure stability of antibodies. For strong and correct correlation between laboratory results and patient records, rigorous specimen labelling as well as chain of custody procedures were utilized. IgM and IgG antibodies for rubella were quantified with a commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kit. Microtiter wells pre-coated with rubella viral antigens are used for the assay. Dilute serum samples were spread in the wells, and incubated for antigen–antibody binding.

Horseradish peroxidase (HRP) conjugated anti-human IgM or IgG antibodies were added after washing for elimination of unbound components.

The solution was added after incubation followed through with washing until blue. This blue color indicates antibody concentration ratio. The reaction was terminated with the addition of sulfuric acid, and the absorbance (450 nm) was measured by a microplate reader. The levels of IgM and IgG were then read in the laboratory in a manner of the manufacturer's cut-offs, where the result is classified as negative, equivocal or positive. The anti-dsDNA antibody levels were determined through quantitative ELISA methods that included plates coated with purified double-stranded DNA.

Serum samples were then diluted and placed into wells to allow binding between dsDNA antigens and corresponding antibodies. After being incubated, unbound proteins were washed away with a fine layer of washing buffer and enzyme-conjugated secondary antibodies were added. Colorimetric detection using TMB substrate was carried out, and absorbance was measured at 450 nm. The concentration (IU/mL) was calculated using a standard calibration curve prepared using the known dsDNA antibody standards. Values >25 IU/mL were detected as significantly elevated and possibly associated with autoimmune activation. All tests had to be performed in duplicate for reproducibility. IQC for all ELISA assays was performed using the appropriate positive controls, negative controls, and calibrators as issued by the kit supplier. Plates with CV >10% between duplicates were repeated. All pipettes were calibrated regularly, and reagents were kept for safe storage. Laboratory staff followed good laboratory practice (GLP) and BSL-2 guidelines. Samples presenting hemolysis, lipemia, or low volume of samples were excluded from analysis. Demographic variables (age, blood group), clinical variables (abortion history), and laboratory results (IgM, IgG, anti-dsDNA level) were rigorously entered into a structured Microsoft Excel sheet and imported into Python (Pandas) to perform processing and statistical analysis. Data cleaned by excluding missing values, correct variable types, outlier. In order to protect confidentiality, each participant was assigned a code name. Statistical interpretation was only undertaken with de-identified data. Data analysis was conducted with Python (NumPy, Pandas, Matplotlib, Seaborn). Descriptive statistics (mean, median SD, minimum, maximum and quartiles), were performed on the major predictors. Visualizations that included histograms of Age, IgM, IgG, and anti-dsDNA; scatter plots of dsDNA against IgM and IgG; box plots with anti-dsDNA levels by abortion category, and bar charts for blood groups were done. Differences between abortion number and anti-dsDNA were measured by group means and variance. Data correlation between dsDNA and rubella markers (IgM, IgG) was examined with scatter plots. On request, statistical significance testing (ANOVA, Kruskal–Wallis, and correlation coefficients) can be done. For hypothesis testing, $p < 0.05$ was considered as a significance threshold.

Results:

In our clinical exam, it was 59 reproductive-age (mean age = 28.63 years) women, equal to population size from birth across this region (range = 13-44 years, heterogeneous sample). In the distribution curve, we are much less likely to omit young people, and older people, and more likely to consider people who were 25 to 35 years old in maximum reproductive age as our sample number, thus we show normal distribution.

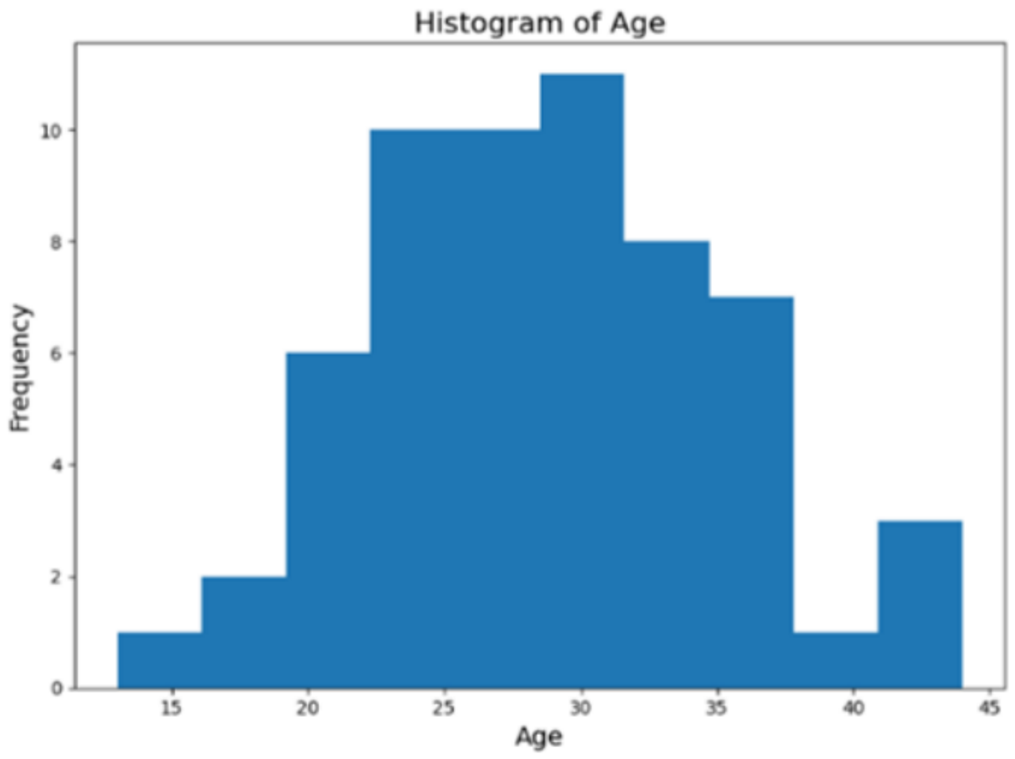

Figure 1: The women's age Histogram

The blood group frequencies showed O+ as the most prevalent group (52.5%), and A+, B+, and O− as the least frequent frequencies, suggesting that the hematological characteristics observed in the Iraqi/Kurdish population (see previous works) were more reliable in general. That said, few were available (AB−, AB+, and B−, etc.), and too few to compare subgroups. The women were clinically stable and had no previous history of documented autoimmune or chronic infectious disease, along with having an acceptable sample size for evaluating immunological markers such as rubella serology and anti-dsDNA activity.

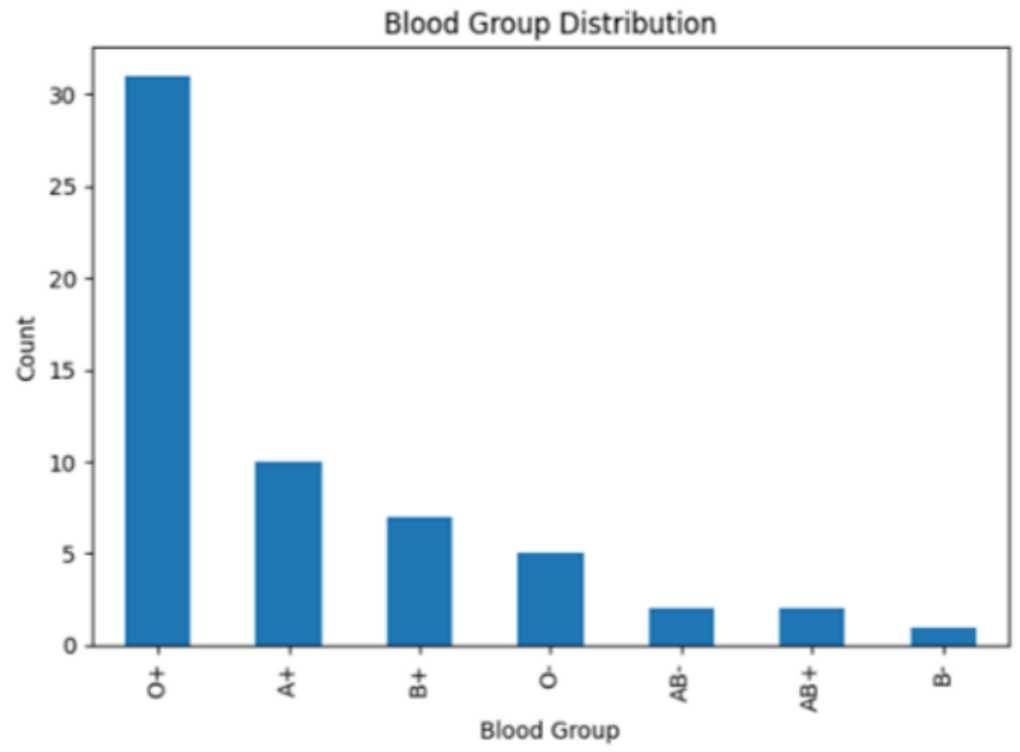

Figure 2: The blood group of the women

Serology indices for the immune responses to rubella revealed a highly heterogeneous immune response from IgM estimates of 0.16-1.91 IU/mL and a mean value = 0.709, inferring that almost all the patients were classified as “non-acute” group for rubella infection and suggesting that they have lower active or recent infection with rubella compared to other groups among these patients. IgMs immeasurable on the

histogram are lopsided to the right (predominantly < 1.0 IU/mL), demonstrating no recent seroconversion for the IgM levels in these patients. In comparison, the IgG values from Rubella ranged from 0.13 to 2.31 IU/mL, suggesting more substantial heterogeneity in immunity of recipients to rubella (mean, 0.849). This inconsistency may be due to variability in previous exposure history, efficacy of vaccinations, immunological susceptibilities, or incomplete immunological memory. Several women had relatively high IgG consistent with childhood exposure or seroconversion in children that may also be associated with immunologic consequences.

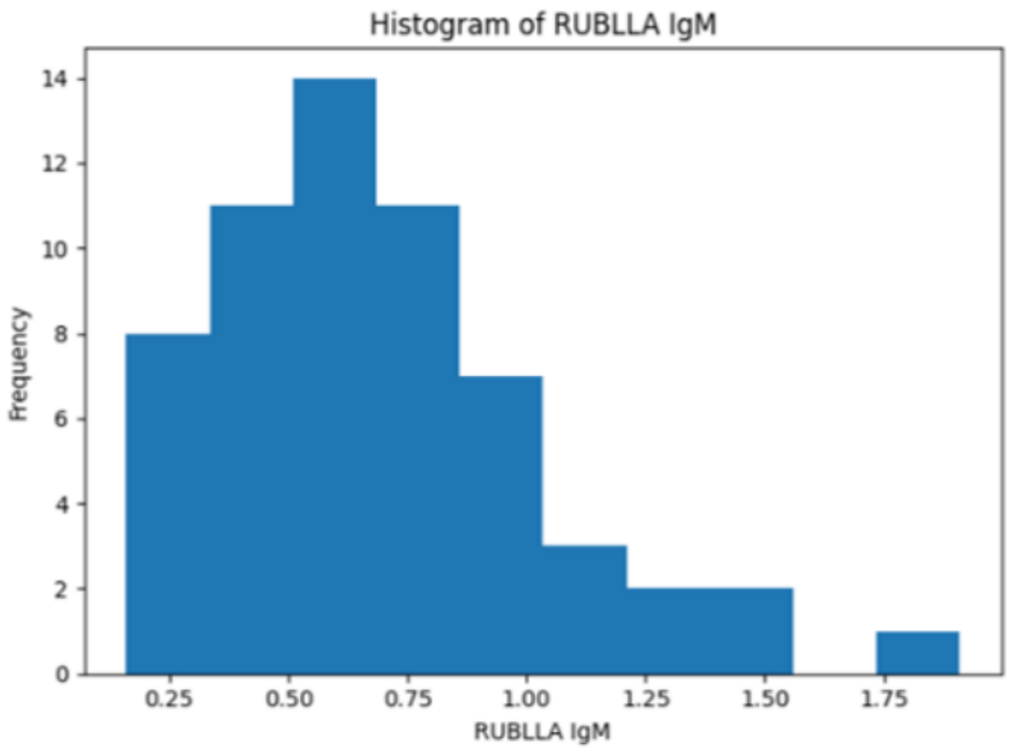

Figure 3: The RUBLLA IgM Distribution

The anti-dsDNA antibody concentrations obtained in the sample distributed very highly across the population, with an average of 8.458 between 0.49 IU/mL and 33.56 IU/mL, indicating that most participants had antibodies that were typical or slightly higher than standard levels, and in a large number with titers >normal levels, due to increased immune activation. The distributions varied widely between all subjects and were in the classically right-skewed direction, but some of the distributions were considerably larger than studies on autoimmune diseases (i.e., SLE). This seems to suggest that abundant anti-dsDNA production promotes an improved immune response in a subgroup of disease conditions. Two subjects tittered for values > 28 IU/mL, suggesting an underdiagnosed autoimmune disease, viral-mediated immune response disturbance, or subclinical inflammatory response. In asymptomatic otherwise reproductive women, the titers would probably be surprising, and the dsDNA titer scales of that size could constitute a basis for studies on the relationship of autoimmunity, past viral exposure, and reproductive endpoints like miscarriage or recurrent abortion.

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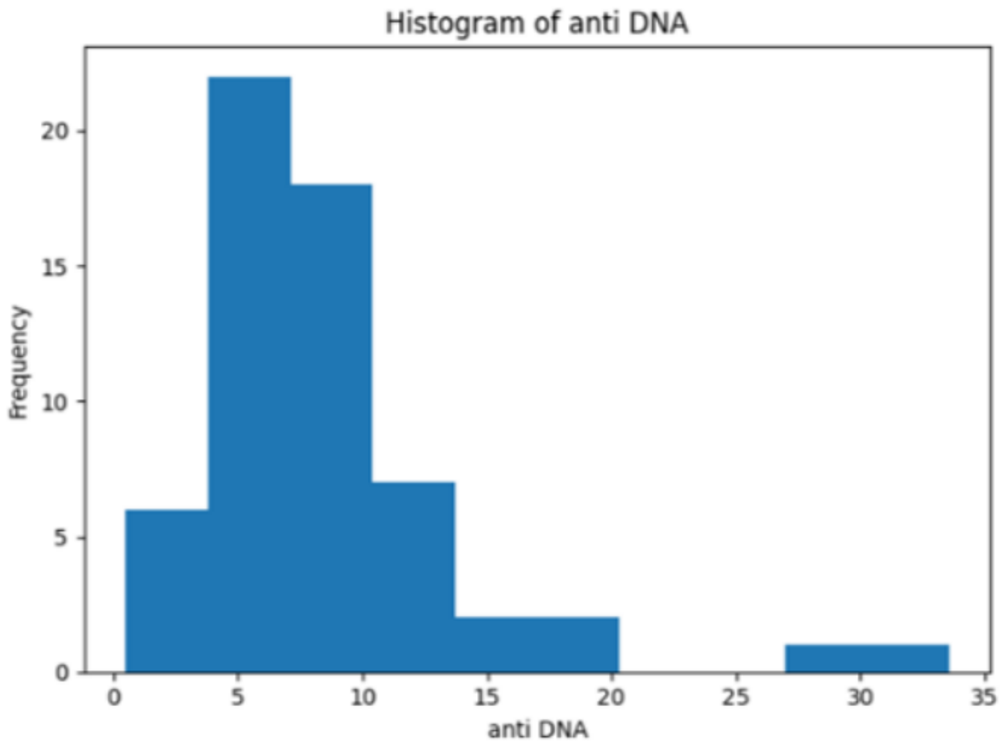

Figure 4: The anti-DNA histogram

Abortion history analysis demonstrated a wide range of pregnancy loss frequency, from 0 to 7 abortions, enabling meaningful comparison of anti-dsDNA antibody profiles across reproductive subgroups. Women with 0 abortions had a mean anti-dsDNA level of 7.15 IU/mL, whereas those with 1 abortion showed a higher mean of 8.30 IU/mL, indicating a modest rise in autoantibody levels early in reproductive complications. More striking elevations were observed in women with 2 (mean 10.49 IU/mL) and 3 abortions (mean 10.14 IU/mL), who also displayed the highest variability and the most extreme outliers (33.56 and 28.24 IU/mL), strongly suggesting the involvement of heightened autoimmune activity in a subset experiencing recurrent pregnancy loss. These findings reflect a possible dose–response relationship, where increasing abortion number is associated with rising autoimmune antibody production, at least up to the third pregnancy loss. Interestingly, women with six and seven abortions did not show elevated autoimmune markers, suggesting that not all recurrent pregnancy loss is immunologically mediated and that other etiological factors likely dominate in cases of very high miscarriage frequency. The trend observed supports a potential immunopathological role for anti-dsDNA in early recurrent abortions and emphasizes the need for further clinical evaluation of autoimmune markers among women with repeated pregnancy loss.

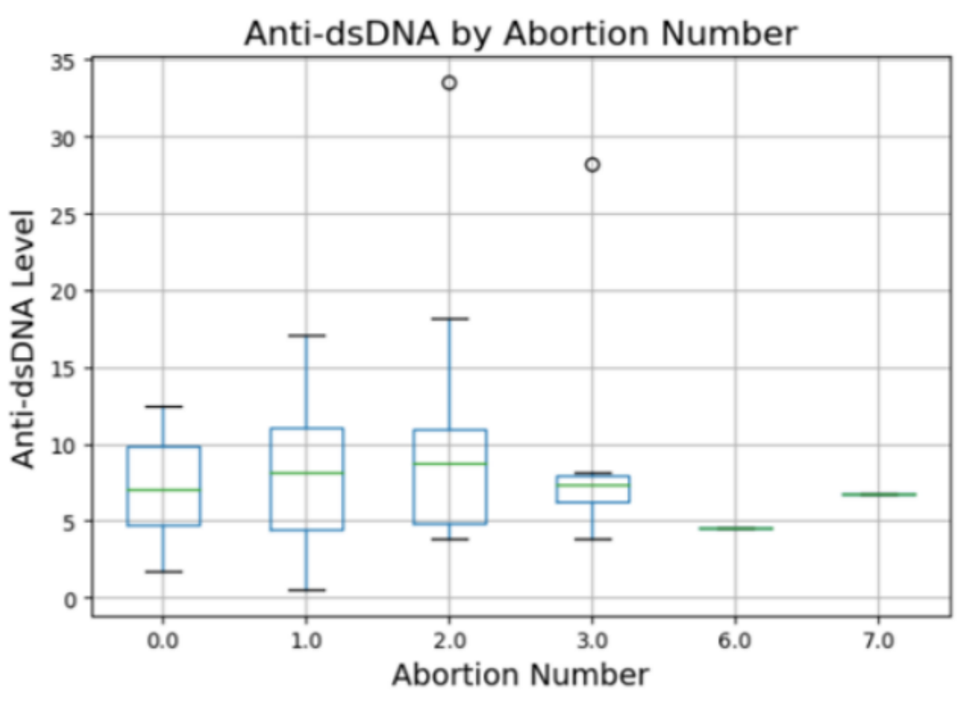

Figure 5: The Anti-dsDNA by Abortion number

Examination of the interplay between rubella immunity and anti-dsDNA antibody levels revealed that Rubella IgM showed no observable relationship with dsDNA titers, as participants with the highest or lowest IgM levels displayed no corresponding changes in autoimmune antibody production. The results confirm that acute rubella infection is not a major driver of dsDNA-mediated autoimmunity in this population. The relationship between Rubella IgG and anti-dsDNA demonstrated a more nuanced pattern, although the overall correlation was weak and non-linear. Scatterplot visualization revealed a distinct cluster of elevated dsDNA levels among women with moderately elevated IgG titers. Our results also suggest that prior rubella exposure may contribute to immunological stimulation or prime the immune system toward autoimmune reactivity; possibly through mechanisms such as molecular mimicry, cross-reactive antibodies, or post-viral immune modulation. While rubella does not appear to be the sole determinant of autoimmune activation, its potential role as a trigger or cofactor in susceptible individuals warrants further exploration, especially in women experiencing repeated pregnancy loss.

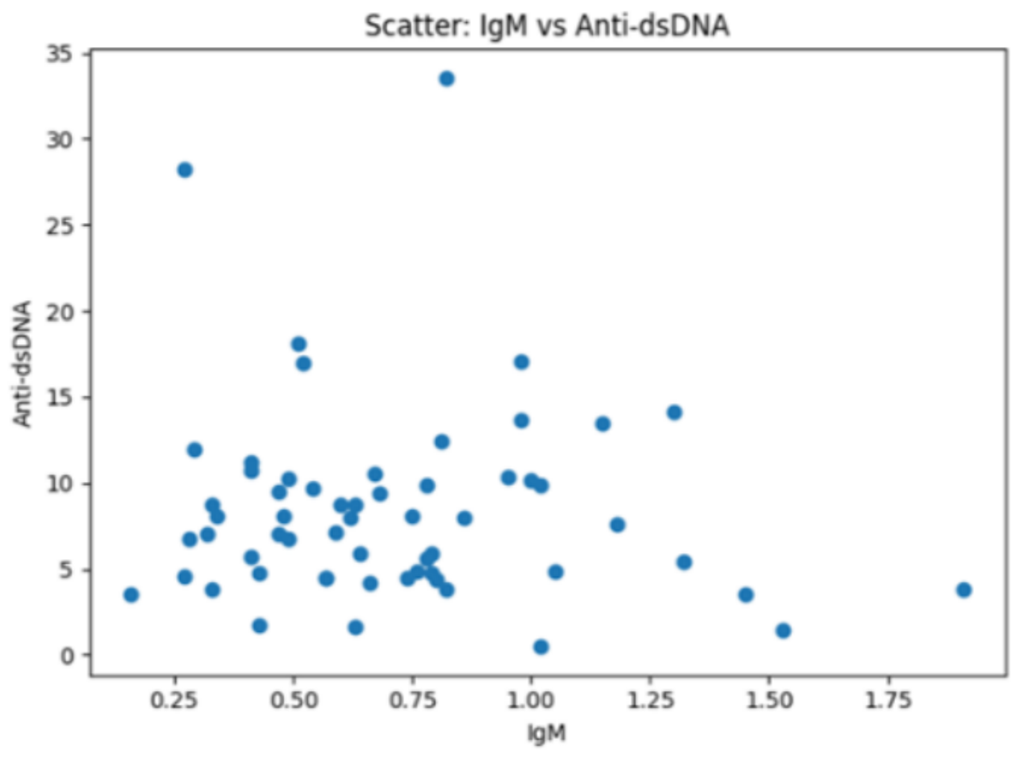

Figure 6: IgM vs Anti-DNA

The results also demonstrate that rubella immunity varies widely in the population, with low IgM values indicating minimal active infection and diverse IgG levels reflecting varied exposure or immunization histories. Anti-dsDNA antibodies showed substantial variability, with several individuals exhibiting levels strongly suggestive of autoimmune activation. A clear upward pattern was identified between the number of abortions and anti-dsDNA levels, particularly in women with two or three abortions, who also exhibited the highest autoimmune outliers. While very high abortion numbers (≥6) did not correlate with elevated dsDNA, this aligns with the understanding that recurrent pregnancy loss is multifactorial and may involve non-autoimmune etiologies.

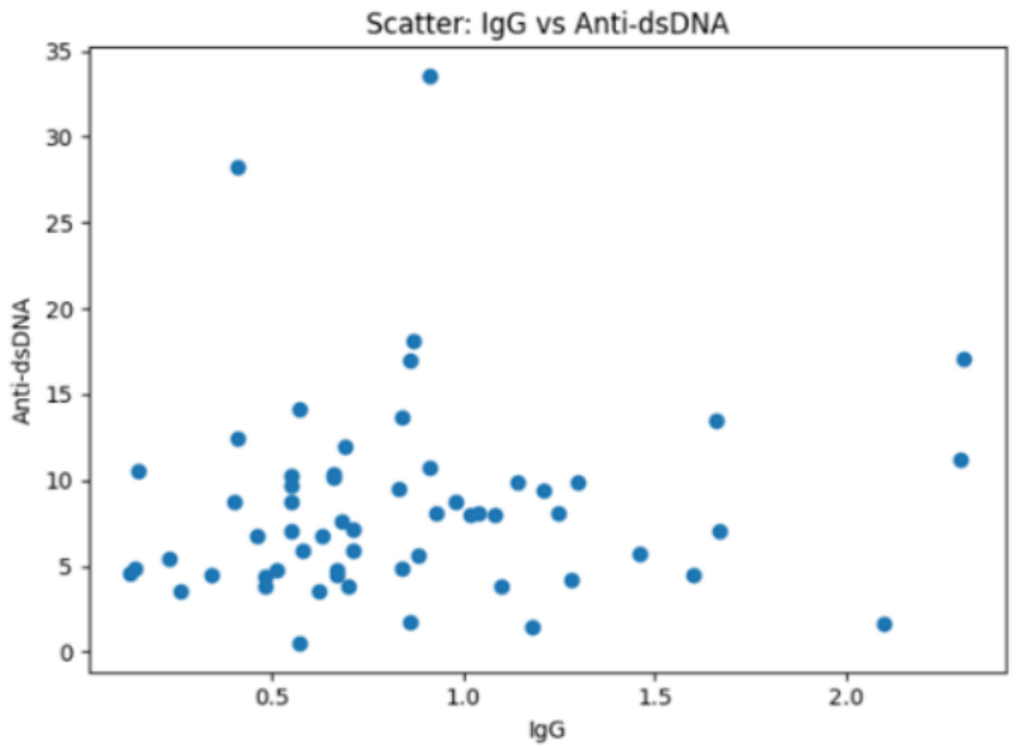

Figure 7: IgG vs Anti-dsDNA

Discussion:

The purpose of this research was to examine the correlation between anti-dsDNA antibodies and autoimmune markers, as well as the correlation between rubella serological status and abortion history in reproductive-aged women. The results of this study add to our knowledge of viral-immune interactions by revealing more features that are important in clinical practice; women in developing countries exhibit a wide range of rubella immunity and anti-dsDNA titers, according to previous research. Higher anti-dsDNA titers were found in females with intermediate levels of IgG, but no correlation was found between IgM and anti-dsDNA. These results provide more evidence that previous rubella exposure might activate the immune system through molecular replication or some other pathway.

Despite the common association between anti-dsDNA and SLE, subclinical elevations have been found in cases of immunological dysregulation caused by viruses and in women who have unfavorable pregnancy outcomes [17]. Autoantibodies can be produced in response to certain viruses, such as rubella, parvovirus B19, or Epstein-Barr virus, through processes like bystander activation, epitope spreading, or molecular mimicry involving viral proteins and human nuclear antigens [6; 18]. Rubella is not as likely as parvovirus or EBV to cause autoimmunity, but many studies have shown that susceptible people can still develop autoimmune reactions after getting the vaccine or contracting the virus naturally. In these cases, autoantibodies like ANA, anti-dsDNA, or antiphospholipid antibodies can even emerge [5; 15]. The new data add to the existing body of knowledge by suggesting the potential for viral-immune interactions within the immunological environment, as a small number of women exhibited elevated dsDNA levels even without a clinically recognized autoimmune disorder. The results show that there is a strong correlation between anti-dsDNA antibody levels and the frequency of abortion. Women with a history of two or three abortions showed the most significant outlier results and elevated mean dsDNA titers, suggesting that reproductive failure may have an autoimmune component. There have been previous studies showing that ANA, anti-dsDNA, and antiphospholipid antibodies are more commonly found in women who have recurrent miscarriages compared to control populations. This finding is in line with those studies [19; 14].

However, antiphospholipid syndrome is generally recognized as the principal

autoimmune cause of miscarriage [20; 21]. Other autoantibodies, such as anti-dsDNA, have been suggested to interfere with implantation, trophoblast proliferation, and placental angiogenesis in recent investigations. Furthermore, unexplained recurrent pregnancy loss is a condition that does not have definitive answers through traditional diagnostic methods; subclinical autoimmunity is being acknowledged as a factor in these cases [7]. It's found no correlation between a woman's history of six or more abortions and an increase in anti-dsDNA levels, suggesting that factors other than autoimmune processes contribute to recurrent high-order pregnancy loss. The higher anti-dsDNA titers observed in the group of women who had had two or three abortions before provide credence to this growing trend. One possible link is the association between a family history of autoimmune diseases and a history of rubella exposure. Some of the ways in which the rubella virus can worsen autoimmune reactions include changing how antigens are presented, triggering localized inflammatory responses, and increasing levels of cytokines such as IFN-α and IL-6. Changes to the immune system have also been shown [22; 23].

Conclusion:

This study found a connection between high levels of anti-dsDNA antibodies and repeated pregnancy loss, particularly in women who had experienced two or three miscarriages, suggesting a possible autoimmune cause. Rubella IgM didn't show any link, while changes in IgG levels might indicate immune system activation in those who are vulnerable. These findings highlight the importance of screening for autoimmune issues in cases of unexplained miscarriage and support further research into how viruses and autoimmune responses interact and affect reproductive health.

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العلاقة بين عدوى الإصابة بالحصبة بمرض الحصبة الألمانية في النساء ومستويات الاجسام المضادة للـdsDNA

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الخلاصة

لقياس ELISA استخدمت منهجية المقاطع العرضية، تم جمع عينة دم من 59 امرأة في سن الإنجاب لتحليلها باستخدام تقنية ، anti-dsDNA ، بالإضافة إلى الأجسام المضادة للـIgGو IgM تركيزات الأجسام المضادة للحصبة الألمانية من النوع ، وبين تركيزات الأجسام المضادة للـIgGو IgM وذلك لتحديد العلاقة بين مؤشرات المناعة ضد الحصبة الألمانية، وتحديدًا للحصبة الألمانية منخفضة بشكل كثير في جميع العينات المأخوذة، مما يشير إلى IgM ؛ كانت مستويات anti-dsDNA تفاوتًا كبيرًا، مما يعكس اختلافات في التعرض المسبق أو المناعة. كانت IgG غياب العدوى الحادة، بينما أظهرت مستويات متغيرة ؛ حيث أظهرت بعض الحالات تركيزات مرتفعة بشكل ملحوظ، مما anti-dsDNA مستويات الأجسام المضادة للـ بين النساء اللاتي أجرين anti-dsDNA يشير إلى نشاط مناعي ؛ ووجدت الدراسة أيضًا زيادة تدريجية في مستويات الـ عمليتين أو ثلاث عمليات إجهاض، وهي مجموعة أظهرت أعلى تركيزات خارجية للأجسام المضادة، مما يشير بقوة إلى ترابط العمليات المناعية في فقدان الحمل المتكرر في مجموعة معينة من النساء؛ أما النساء اللاتي لم يخضعن عمليات إجهاض أو ، مما يدل على أن فقدان الحمل المتكرر الحاد يرجع على الأرجح إلى dsDNA أكثر فقد أظهرن مستويات طبيعية من الـ ، ولكن تركيز الأجسام المضادة للـanti-dsDNA للحصبة الألمانية مرتبطة بـ IgM أسباب غير مناعية. لم تكن مستويات قد يشير إلى تأثيرات .IgG المرتفع بشكل معتدل لدى بعض النساء اللواتي أظهرن مستويات مرتفعة نسبيًا من dsDNA تحفيزية للمناعة نتيجة التعرض المسبق للحصبة الألمانية. تدعم هذه النتائج الفكرة القائلة بأن النشاط المناعي، بدلاً من العدوى الفيروسية النشطة، قد يساهم في فقدان الحمل، مما يبرز الحاجة إلى دمج فحص المناعة الذاتية، بما في ذلك اختبار الأجسام ، في التقييم السريري للإجهاض غير المبرر أو المتكرر. بالإضافة إلى ذلك، تسلط الدراسة الضوء anti-dsDNA المضادة للـ على أهمية مراقبة مناعة الحصبة الألمانية وتقترح ضرورة إجراء أبحاث مستقبلية حول التفاعلات الفيروسية–المناعية في سياق صحة الإنجاب

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الكلمات المفتاحية: العمر، الحمض النووي مزدوج الشريطة، النساء، الحصبة الألمانية، العدوى