

Seroprevalence of Antinuclear Antibodies, Antibrucella Antibodies, and Hepatitis B Surface Antigen in Women with Recurrent Abortion

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Abstract

Background: Recurrent abortion is a globally common problem. Many factors play a crucial role in the pathogenesis of abortion such as antinuclear antibodies (ANA), antibrucella antibodies and hepatitis B surface antigen (HBsAg). **Objectives:** The study objective was to estimate the serum levels of antinuclear antibodies (ANAs), antibrucella antibodies, and hepatitis B surface antigen among recurrent aborting women. **Materials and Methods:** One hundred and twenty women were enrolled in this prospective case–controlled study from Maternity Hospital, Erbil City, Iraq. The study included sixty patients with a history of three and more attacks of previous abortions and sixty healthy pregnant women. All were screened for ANA-IgG, antibrucella antibodies, and hepatitis B surface antigen (HBsAg) in serum. **Results:** It was noted that 15/60 (25%) patients and 6/60 (10%) healthy controls were positive for ANA-IgG with a mean concentration of 6.2 ± 0.4 IU/ml in the patient group and 0.75 ± 0.325 IU/ml in the healthy controls with statistical significance at $P \leq 0.05$. The distribution for ANA with antibrucella and HBsAg in patient group was as follows: 12/15 (80%) and 6/15 (46.15%), respectively, among the seropositive ANA patients with a high significance at $P < 0.01$ for antibrucella and a significance at $P < 0.05$ for HBsAg. **Conclusions:** ANAs constitute an important cause of recurrent mid-trimester abortion in women. Furthermore, there is growing clues of the pathogenicity of *Brucella* organisms and hepatitis B virus, especially as agents of adverse pregnancy.

Keywords: Antibrucella antibody, antinuclear antibody, hepatitis B surface antigen, recurrent abortion

INTRODUCTION

Pregnancy and linked disorders in women of reproductive age group are common.^[1] Unwanted recurrent pregnancy loss is one of the known problems in medicine. Depending on the time of the event, this phenomenon refers to the two terms, abortion (end of pregnancy from the beginning of gestation to 20 weeks) and fetus loss (end of pregnancy from 20 weeks).^[2] The causes of abortion are different, and many factors such as hormonal, genetics, anatomic, systemic hypertension, infections, diabetes, and hyperthyroidism have been well known to cause this problem.^[3] Immunological agents are shared in recurrent abortion cases,^[4] and they are the most noticeable causes of abortion.^[5]

Antinuclear antibodies (ANAs) in women with repeated miscarriage have been announced. The existences of mild to high titers of these antibodies elucidate an autoimmune status that can endanger the health of the fetus in pregnant women.

ANAs are a particular category of autoantibodies that have the capacity of binding and destroying certain structures within the nucleus of the cells.^[6] Intracellular bacteria that grow either poorly or not in the least media used habitually to detect human pathogens could be the etiological agents of these obstetric conditions. Increased rates of recurrent pregnancy loss, premature delivery, and intrauterine infection with fetal death have been described among pregnant women with clinical evidence of brucellosis.^[7,8] Infection with hepatitis B virus (HBV) in pregnant women may be a threat for each mother and fetus. Viral hepatitis (HBV) infection is one of the

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major diseases of humankind that has shown to cause a serious public health problem. Hepatitis B surface antigen (HBsAg) expression has also been found in cells of the ovarian follicle or placental capillary epithelial tissue.^[9]

This study aimed to determine the serological levels of ANAs, antibrucella antibodies, and hepatitis B surface antigen among recurrent aborting women.

MATERIALS AND METHODS

The study protocol and sampling

This study was conducted from September 2017 to September 2019, at Maternity Hospital, Erbil City, Iraq. Investigations for the hormonal assays and infections (*Cytomegalovirus*, rubella, and toxoplasmosis) were negative. The inclusion criteria were patients with a history of three or more consecutive early pregnancy losses and patients with a history of hepatitis or brucellosis involved. One hundred and twenty women (sixty aborted and sixty healthy controls) were enrolled in this case-control study. The controls were women with normal pregnancy who were para 3 and gravida 4. The diagnosis was approved according to the clinical criteria and partial thromboplastin time.^[10]

The exclusion criteria were the following: a history of uterine anomalies; diabetes mellitus; thyroid disease; aspirin, heparin, antibiotics or corticosteroid intake; embryo anomalies; chronic systemic disease including lupus, autoimmune diseases, hypertension, asthma, and cardiopulmonary diseases; and a previous history of asymptomatic urinary tract infection.

Ethical consideration

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. The Ethics Committee of Erbil Polytechnic University, Iraq/Erbil Medical Technical Institute, approved the protocol. Informed consent was obtained from all the patients. The strictly selected patient group had a history of three or more consecutive early pregnancy losses.

Analytical methods

A 5-ml aliquot of blood was taken from each patient. The separated serum was stored at -30°C until analyzed for ANA, anti-*Brucella* antibodies, and HBsAg. The serum samples were also subjected to enzyme-linked immunosorbent assay (ELISA)-specific ANA as described by the manufacturer (Diagnostic Automation, Woodland Hills, California, USA). ELISA test system is designed to detect IgG class antibodies to a variety of common nuclear antigens in human sera. The result was referred as negative if optical density ratio negative specimens ≤ 0.90 , equivocal specimens = $0.91-1.09$, and positive if it is ≥ 1.10 , with cutoff OD = 0.198 . Index values or OD ratios = specimen OD/cutoff OD calculated as suggested by the manufacturer; the sensitivity and specificity of the kit are 96.6% and 100%, respectively.

Serum samples were tested for anti-*Brucella* antibodies using the Rose Bengal plate test (RBPT) a direct enzyme-linked

immunosorbent assay (ELISA; JOVAC, Amman, Jordan). Positive samples were confirmed using the complement fixation test (CFT) (JOVAC, Jordan). In the case of CFT, serum samples with 17 IU/mL antibody or higher were regarded as positive. Titers^a = 0 were under the cutoff value of 17 IU/mL and hence negative. Titers $\geq 1:10$ were above the cutoff value and hence positive. As suggested by the manufacturer, the sensitivity and specificity of RBPT were 89% and 97%, respectively. CFT has a sensitivity of 88.1% and a specificity of 100%. Positive and negative control sera were used.

The ACON HBsAg *in vitro* diagnostic test strips were used for the test (ACON Laboratories, USA). With the arrows pointing downward, the ACON strip was immersed into the serum for 10–15 s. The maximum line (MAX) on the strip was observed, and the strip was then placed on a nonabsorbent surface, the timer was set for 15 min, awaiting the red lines to appear. Two distinct red lines appeared, one on the control region (C) and the other red line on the test (T) region. The intensity of the red line varied depending on the concentration of the HbsAg in the specimen. Therefore, any shade of red line in the test (T) region was considered positive, and one red line on the control (C) region and no shade of red color on the test (T) region were considered as negative. The test was invalid if the control line failed to appear and was discarded.

Statistical analysis

Data were expressed as mean $\pm$ standard deviation, percentages, and ranges as appropriate. Comparisons were made using Student's *t*-test and Chi-square test using standard equations in calculator manually. The results were reported with $P \leq 0.05$ as the accepted level of significance.

RESULTS

Table 1 illustrates the distribution of ANA in the patient and control group. In the patient group, 15/60 (25%) patients had a positive ANA-IgG elevation with a range and mean of 0.25–4.4 IU/ml and 6.2 ± 0.4 IU/ml, respectively. In the control group, 6/60 (10%) controls had a positive ANA elevation with a range and mean of 0.5–0.9 IU/ml and 0.75 ± 0.325 IU/ml, respectively. The difference in the number of individuals with positive ANA and the means between both groups was highly significant by using Chi-square and the Student's *t*-test, respectively ($P \leq 0.01$).

Table 2 shows that from 15 ANA-positive recurrent aborting women, 12 (80%) had a positive elevation of antibrucella antibodies, while the rest 3 (20%) women had negative antibrucella antibodies, whereas in the rest 45 ANA-negative recurrent aborting women, 3 (6.67%) had positive antibrucella antibodies. The rest 42 (93.33%) women had normal levels of antibrucella antibodies. Chi-square test showed a highly statistically significant difference regarding the distribution of ANA in both groups ($P \leq 0.01$).

Table 3 shows that from 15 ANA-positive recurrent aborting women, 6 (40%) had a positive elevation of HbsAg, while

Table 1: Distribution of antinuclear antibody in both patient and control groups with their mean±standard deviation and the range of concentrations observed

Groups	ANA positive (%)	ANA negative (%)	Total	Mean±SD (IU/ml)	Range (IU/ml)
Patients	15 (25)	45 (75)	60	6.2±0.4	0.25-4.4
Control	6 (10)	54 (90)	60	0.75±0.325	0.5-0.9
Total	21	99	120		

The Chi-square statistic is 4.6753. The P value is 0.030599. This result is significant at $P<0.05$. Student's *t*-test revealed that this difference is considered to be extremely statistically significant $P\leq 0.01$. *t*: 29.5592, df: 19. Negative specimens ≤ 0.90 , equivocal specimens: 0.91-1.09, positive specimens: ≥ 1.10 , Cutoff OD: 0.198. ANAs: Antinuclear antibodies, SD: Standard deviation, OD: Optical density

Table 2: Distribution of antibrucella antibodies in both antinuclear antibody-positive and antinuclear antibody-negative patient groups

Patient group	ANA positive		ANA negative		Total (%)
	n (%)	CFT titers ^a	n (%)	CFT titers ^a	
Positive antibrucella	12 (80)	1:2560, 1:180, 1:1280, 1:160, 1:320, 1:80, 1:1280, 1:320, 1:2560, 1:80, 1:320, 1:640	3 (6.67)	1:320, 1:80, 1:320	15 (25)
Negative antibrucella	3 (20)	0	42 (93.33)	0	45 (75)
Total	15		45		60

The Chi-square statistic is 32.2667. The P value is <0.00001 . The result is significant at $P<0.01$. ^aTiters: 0 were under the cutoff value of 17 IU/mL and hence negative. Titers $\geq 1:10$ were above the cutoff value and hence positive. ANAs: Antinuclear antibodies, CFT: Complement fixation test

Table 3: Distribution of hepatitis B surface antigen in both antinuclear antibody-positive and antinuclear antibody-negative patients

Patient group	ANA positive (%)	ANA negative (%)	Total (%)
Positive HbsAg	6 (46.15)	9 (19.15)	15 (25)
Negative HbsAg	7 (53.85)	38 (80.85)	45 (75)
Total	13	47	60

The Chi-square statistic is 3.9607. The P value is 0.046574. The result is significant at $P<0.05$. HbsAg: Hepatitis B surface antigen, ANAs: Antinuclear antibodies

the rest 9 (60%) had a negative HbsAg. Among the rest of the 47 ANA-negative recurrent aborting women, 9 (20%) had positive HbsAg, while the rest of the 36 (80%) women had normal levels of HbsAg. Chi-square test showed a statistically significant difference regarding the distribution of ANA in both groups ($P\leq 0.05$).

DISCUSSION

In this study, ANAs were found in 15 (25%) aborted women with RPL and 6 (10%) in the control group, which had attended the maternity hospital in Erbil city. This result is in parallel to the studies by Anupriya *et al.*,^[11] Garcia *et al.*,^[12] and Nakatsuka *et al.*,^[13] in which the prevalence of ANA among women with RPL was 30%, 30%, and 43.5%, which were higher than that of the control woman group, i.e., 8.3%, 6.6%, and 22.4%, respectively. Molazadeh *et al.*^[14] had reported a high prevalence of ANA (13.21%) among patients with both explained and unexplained pregnancy losses. Sakthiswary *et al.*^[15] concluded that the existence of ANAs may predict a higher chance of pregnancy loss in cases with a history of previous recurrent pregnancy loss.

Shoenfeld *et al.*^[16] found a higher prevalence of ANAs in patients with autoimmune disease.

ANAs may interfere with the formation and maturation of placenta, which would eventually lead to an early fetal loss. Yielding of antibodies versus histone proteins may procure to the brisk of the autoimmune process in mother and thereby end up in abortion. ANAs are a specific class of autoantibodies that tend binding and destroying certain structures within the nucleus of the cells.^[6] The mechanism by which ANAs cause pregnancy loss is not known well, Antinuclear antibodies may interfere with the formation and maturation of placenta which would eventually lead to an early fetal loss. Yielding of antibodies against of histone proteins may procure to the brisk of the autoimmune process in mother and thereby end up in abortion.^[17]

Tables 2 and 3 show the distribution of antibrucella antibodies and HBsAg in ANA +ve and ANA -ve patient group. The statistical analysis clarified that there was a highly statistically significant difference regarding the distribution of antibrucella ($P\leq 0.01$) and statistically significant difference regarding the distribution of HBsAg in both groups ($P\leq 0.05$).

Overall, the prevalence of *Brucella* antibodies and HBsAg among ANA +ve and ANA -ve pregnant women was 15 (25%). This finding is in close agreement with that of larger retrospective studies investigating the consequences in pregnant women with an established *Brucella* infection, which have elucidated a vigorous link between infection and an increased incidence of adverse obstetric outcomes.

These larger case studies have been conducted in the Middle East (Turkey, Saudi Arabia, and Iran), South America (Peru), and Africa (Egypt, Rwanda), with spontaneous miscarriage rates ranging from 18.6% to 73.3%.^[7,8,18-19] A study in the UK conclude that only two of 438 pregnant women exhibited

antibrucella antibodies. These patients were from Sudan with a history of spontaneous abortion or intrauterine fetal death associated with seropositivity for brucellosis.^[20]

Brucellosis is habitually misdiagnosed, or at the best diagnosed indiscriminately; therefore, physicians in both endemic and nonendemic areas should be aware of brucellosis and consider this disease in the differential diagnosis of increased rates of spontaneous abortion, premature delivery, and intrauterine infection with fetal death.^[21,22]

Several studies conducted over the last 30 years had demonstrated a correlation between increased rates of adverse pregnancy outcomes with infection by certain microbial agents.^[9] Trophoblasts are cells that feed the embryo and ultimately develop into a portion of the placenta. *Brucella* has been proven to be capable of replication in trophoblasts, which could intervene with their propagation and dissemination, possibly linked to their leverage on laminin-receptor-1 (laminins are extracellular proteins that are a fundamental portion of the structure of all tissues).^[23,24] Raschi *et al.*^[25] showed that anti- β 2GP1 antibodies induce an endothelial signaling cascade comparable with that activated by lipopolysaccharides (LPS) through the involvement of the toll-like receptor, TLR-4. Shoenfeld *et al.*^[16] speculated that β 2GP1 alone or complexed with its own endothelial cell membrane receptors might interact with TLRs and that anti- β 2GP1 antibody recognizes the molecule might crosslink it with TLRs, eventually triggering the signaling cascades and consequently increased synthesis and secretion of pro-inflammatory cytokines.

Evidence suggests that the putative β 2GP1 phospholipid binding site might be involved in the binding to anionic endothelial cell structures, such as heparin sulfate, and annexin A2, the receptor for plasminogen/tissue plasminogen activator.^[26] Annexin A2 has been suggested to require TLR-4 as a co-receptor to signal because annexin A2 binds β 2GP1 with high affinity but does not display any transmembrane protein.^[26,27] TLR-4 is a key ingredient of the innate immune response and can realize specific microbial products, including LPS. As they are transmembrane proteins, all TLR family members proceed as effective receptors, eligible to boost a prompt inflammatory response after their interaction with specific ligands.^[27]

A two-hit hypothesis has been suggested to explain the common clinical observation that APL might be persistently present even though thrombotic events occur only occasionally: APL (first hit) increases the thrombophilic risk, and clotting occurs in the presence of another thrombophilic condition.^[28]

Fischetti *et al.*^[28] hypothesized that the involvement of TLRs by microbial structures combined with that mediated by anti- β 2GP1 antibodies might synergistically contribute to the second hit that triggers the clotting event. Such a clear prospect stage is in line with a recent *in vivo* experimental model. Human anti- β 2GP1 IgG infused into a naïve rat does not significantly

affect the mesenteric microcirculation, but the same IgG fractions trigger clotting if a primary pro-inflammatory factor, such as LPS, is present. These findings suggest a role for infectious agents as a second hit and the involvement of receptors of innate immunity at the same time.^[29]

In this study, HBsAg in ANA +ve and ANA -ve patient group was found in 15 (25%) of the aborted women with RPL, who had attended the maternity hospital in Erbil city. This result is in consonance with larger retrospective studies investigating the outcomes of pregnant women with a confirmed HBV infection, which have elucidated a robust association between infection and an increased range of inverse obstetric outcomes and the assumption of a baseline miscarriage risk of 5%.^[30]

Gregorio *et al.*^[31] investigated the effects of interferon- α treatment on the prevalence of a panel of autoantibodies in HBV infection and concluded that autoantibodies are common in HBV infection without being influenced by interferon- α . Another multicentric retrospective study found that 15% of chronic hepatitis B patients were positive for at least one autoantibody that was influenced by anti-HBe antibody positivity and that the HBV genotype had no relationship with extrahepatic manifestations.^[32] Shantha *et al.*^[33] concluded that chronic HBV infection may induce autoimmune hepatitis (AIH) type I. The characteristic laboratory feature of AIH, although not universally present, is hyper-gammaglobulinemia. This nonspecific response is associated with the presence of circulating autoantibodies against ANA which had been proposed by Bellary *et al.*,^[34] Mc Farlane *et al.*,^[35] Manns and Obermayer-Straub,^[36] and Clifford *et al.*^[37] The study by Wong *et al.*^[38] did not disclose a negative influence of HBV infection on the path of pregnancy. It may be linked with a high concentration of estrogens; endogenous antioxidants, which prohibit liver fibrosis; hepatocyte apoptosis; as well as Kupffer cells in the liver.^[39]

Although there are many studies regarding hepatitis C virus (HCV) infections complicated by autoimmune diseases and autoantibodies, few reports are available on HBV-associated autoimmunity. Autoimmunity and autoimmune diseases are frequently present in HBV and HCV infections.^[40] Although the prevalence and clinical significance of nonorgan-specific autoantibodies (NOSAs), especially anti-ANAs, smooth-muscle antibodies (SMA), and anti-liver kidney microsomal type 1 antibodies, have been well documented in HCV infections, this area is still a subject of debate.^[41] For example, the relationship between autoantibodies and some clinical features of viral hepatitis is unclear. Irrespective of whether the virus-induced production of autoantibodies is an epiphenomenon during the progression of viral hepatitis or a contributor to hepatocellular damage, the putative mechanisms for producing autoantibodies in viral hepatitis are considered to be mainly attributable to molecular mimicry^[42] or polyclonal B cell activation.^[43] Current theories of the mechanism of virus-induced autoimmunity fall into three categories which are not mutually exclusive: (a) auto-antigen

modification, (b) disturbance of host immune mechanism involved in the control of autoreactivity, and (c) molecular mimicry.^[44]

In genetically susceptible individuals, auto reactions may continue despite successful elimination of the virus. Plotz^[45] has proposed an alternative form of molecular mimicry involving the production of anti-idiotypes against virus-neutralizing antibodies. As the mirror images of the latter, these anti-idiotypes would resemble the viruses and combine with the complementary virus receptors on host cells, thereby acting as autoantibodies that could participate in tissue-damaging immune reactions. This observation contrasted with certain previous chronic hepatitis C findings, supporting the notion that ANA is associated with more advanced cirrhosis or the severity of liver disease,^[46,47] whereas this observation was in agreement with the findings from other reports.^[48,49]

The extent and outcomes of HBV infection are largely dependent on the quality and diversity of the induced immune responses. Patients with chronic HBV infections tend to develop an activated humoral response with type 2 T helper cells producing interleukin (IL)-4, IL-5, and IL-10, which promote antibody production rather than viral clearance.^[50] Similarly, both AIH and primary biliary cirrhosis (PBC) are characterized by immune-mediated injury to parenchymal liver cells and biliary ducts, respectively, and thus related NOSAs (such as ANA, SMA, and AMA) play a crucial role in the accurate classification of AIH and PBC.^[51]

CONCLUSIONS

High serum ANAs are not uncommon in women with unexplained recurrent miscarriage, suggesting the possible role of an autoimmune disorder on abortion, at least in a subgroup of patients. Maternal HBV carrier status and brucellosis may be independent risk factors for miscarriage, and careful surveillance is warranted. Searching for and detecting asymptomatic bacteria and viruses may be of benefit for preventing spontaneous abortions related to high ANA levels. This study reflects the clinical reality of pregnant women and indicates that HBV carrier status and brucellosis confer substantially increased risk for miscarriage. More importantly, this type of study may provide valuable clues to guide medication and treatment regimens.

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Conflicts of interest

There are no conflicts of interest.

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