

# The Occurrence of Autoantibodies in Sera of Healthy Pregnant Women

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## Summary

Autoantibodies have been known to be detected during pregnancy. The occurrence of autoantibodies during pregnancy was studied in a group of 146 healthy pregnant women from Jan - March 1995. Serum samples were tested for antinuclear (ANA), anti-ds DNA, anti-mitochondrial, anti-smooth muscle and anti-parietal cell antibodies employing the technique of indirect immunofluorescence. Sera from 66 non-pregnant women were used as controls. Among the pregnant group, 2 (1.4%) were found to have ANA positivity in comparison to none in the control group. This difference was found to be not statistically significant. Only 1 (0.7%) was positive for anti-mitochondrial antibody in the pregnant group compared to one in the control group ( $p>0.05$ ). However, anti-ds DNA, anti-smooth muscle and anti-parietal cell antibodies were not detected in both groups. All those positive for autoantibodies were in their 2nd trimester. When these cases were followed-up at the end of their pregnancy, none had complicated pregnancies nor infant abnormalities. Our findings suggest that (a) the occurrence of autoantibodies in pregnant women was not significantly different from non-pregnant controls and that (b) maternal autoantibodies did not appear to cause complications during pregnancy or infant morbidity.

**Key Words:** Antinuclear antibodies, Autoantibodies, Antimitochondrial antibody, Antismooth muscle antibodies, Antiparietal cell antibodies, Anti-ds DNA antibodies, Pregnancy, Immunology

## Introduction

Serum antinuclear antibodies (ANA) are associated with the diagnosis of systemic lupus erythematosus (SLE) and other autoimmune diseases<sup>1</sup>. They may also be found in the elderly<sup>2</sup>, normal individuals<sup>3</sup>, relatives of those with SLE<sup>4</sup> and also in pregnancy. Previous studies have examined ANA in patients with a known autoimmune disorder or those with complications during pregnancy. However, few studies have examined its occurrence in normal pregnant women and reported frequencies ranging from 1-53%<sup>5,6</sup> have been documented. Differences in results recorded may be due to different nuclear antigen substrates used and variations in definition of what level of ANA constitutes a significantly positive result. In addition, time after conception, when specimens were taken, the

varied number of control subjects being compared to, and their parity were also contributing factors.

When serum ANA is detected in the normal pregnant woman, physicians are faced with a dilemma in interpreting its significance. Thus a baseline study to determine the occurrence of ANA and other autoantibodies in the normal pregnant individuals is needed to prevent misinterpretation of results. Positive samples were also studied to establish their correlation with the outcome of pregnancy.

## Materials and Methods

### Subjects

One hundred and forty-six healthy pregnant women

attending the antenatal clinics of Kuala Lumpur City Hall from January 1995 - March 1995 were enrolled in this study. Their mean age was 28.6 years (ranging from 20 to 41). Their parity ranged from 0 to 7. They included subjects of the different races in the community who had no clinical features of autoimmunity or any other chronic diseases. It was observed that most of them came for their first visit during their second or third trimesters of their pregnancy. Their clinical details were obtained after their expected date of deliveries and enquiries were made on the course and outcome of their pregnancies.

### Controls

Sixty-six non-pregnant females with no history of autoimmunity or chronic illnesses volunteered to serve as controls. Their age ranged from 20 - 41 years.

### Methods

Seven mls of blood was obtained from each subject in addition to their routine investigation taken at their first visit. Five mls of blood was obtained from the control group. Serum samples were taken to the laboratory, centrifuged and stored at -20°C until ready for testings.

### Detection of ANA

The substrate used was cryocut sections from mouse liver (4µm thick). Serum samples were screened at a starting dilution of 1:10 with Phosphate buffered saline. This was pipetted to the substrates and left to incubate in a humid chamber for 30 minutes at room temperature. They were then washed gently with 3 changes of PBS for 20-30 minutes after which a drop of Fluorescein isothiocyanate-conjugated rabbit polyvalent antiserum to human immunoglobulin (Behringwerke, Marburg, W. Germany) was pipetted onto the slides and left to incubate for another 30 minutes. The washing step as previously done was repeated. Intensity of fluorescence was read with a Leitz Ortholux II fluorescence microscope. Fluorescence at a dilution of 1:20 was taken to be positive. Procedure was repeated on positive sera in doubling dilutions.

### Detection of anti-ds DNA

Samples that were positive for ANA were screened for anti-ds DNA using *Crithidia luciliae* as substrates

(Behringwerke, Marburg, W. Germany) using an immunofluorescence method<sup>7</sup>. Fluorescent of kinetoplast at a serum dilution of 1:10 or greater was regarded as positive.

### Detection of AMA, SMA, APC antibodies

All samples were assayed for presence of anti-mitochondrial (AMA), anti-smooth muscle (SMA) and anti-parietal cell antibodies (APC) employing the method of IF using composite sections of mouse liver, kidney and stomach as substrate. Screening dilution was started at 1:10 and fluorescence at 1:20 was taken to be positive.

### Statistical analysis

All data was entered and analysed using a statistical software, SPSS. The test of significance was determined using  $\chi^2$  analysis and Fisher's exact test where appropriate. A p value of < 0.05 was taken to be significant.

### Results

A total of 146 healthy pregnancy women were enrolled in this study. There were 112 (76.7%) Malays, 19 (13%) Chinese and 9 (6.2%) Indians while the rest were of other races. Their ages ranged from 20-41 years with a mean age of 28.5 years +/- 5. Seven (4.8%) women were in their first trimester, 103 (70.5%) in their second and 36 (24.7%) in their third trimester. Two subjects (1.4%) were positive for ANA (1+ or more at a dilution of 1:20) compared to none among the 66 age-matched controls ( $p>0.05$ ). The pattern of ANA observed was speckled in both cases. When the positive samples were further tested in doubling dilutions, they did not show any fluorescence. One of them was a 22-year-old Malay nulliparous woman who came at her second trimester while the other was a 41-year-old Malay women with a parity of four in her second trimester.

None was detected positive for anti-ds DNA antibodies. Only one individual (0.07%) was found to be positive for AMA at a dilution of 1:20. This was a 20-year only Malay nulliparous woman in her second trimester. The AMA was positive in 1.5% of the control group ( $p>0.05$ ). None was detected positive for APC antibodies and SMA in both the pregnant and nonpregnant groups.

## Discussion

Immunological changes are known to occur within the immune system during pregnancy<sup>8</sup>. Altered tolerance of the maternal immune system towards the foetus may be the result of autoantibodies in women's sera leading to abortion and may occur at all stages of pregnancy. This has inspired several people to study the different possible immunological changes taking place during the process of pregnancy. The fact that autoimmune disorders improve during pregnancy<sup>9</sup>, and the preponderance of autoimmune disease in women<sup>10</sup> suggest that hormonal factors may have an effect on the immune system.

The occurrence of ANA in normal pregnancy has led several investigators to look into its effect on the outcome of the pregnancy. ANA in normal pregnancy has been variously reported to range from 1-53%<sup>5,6</sup>. This study was carried out not only to determine the occurrence of autoantibodies among healthy pregnant women but also to associate the findings with the outcome of the pregnancies.

There has been little agreement on the frequency of autoantibodies, whether it is increased or decreased<sup>11,12</sup> in pregnancy. Polishuk *et al*<sup>5</sup> found that 53% of normal pregnant women had ANA while only 1% of non-pregnant controls were found positive using human foetal thyroid as substrate. Hess and Baum<sup>6</sup> found only 1/147 had ANA using rat liver as substrate. Farnam *et al*<sup>13</sup> found an ANA frequency of 10.7% among 214 pregnant women at titres of 1:20 and concluded that there was no significant difference in ANA positivity in pregnancy compared to 2% in non-pregnancy controls using mouse kidney sections. Rosernberg *et al*<sup>14</sup> using HEp2 cells found 10% ANA positivity at 1:80 among 100 pregnant women while Kiuttu *et al*<sup>15</sup> found a frequency rate of 5.2% at a titre of more than or equals to 1:20 using McCoy cells but anti-ds DNA antibody was not detected. However, these were refuted by Zurier<sup>16</sup> who did not find any ANA among his group of pregnant women. Ostensen *et al*<sup>17</sup> reported no difference in the frequency of ANA between both groups (19.6% vs.15%). However, Patton *et al*<sup>18</sup> using mouse liver, stomach and kidney sections found 46.2% among 84 pregnant women positive at a titre of 1:20 compared to 0.5% of his controls. This was noted to

be insignificant. This study also looked into the occurrence of AMA, SMA, and APC and found the frequency with which at least one autoantibody was detected in the pregnant and control groups did not differ significantly.

Hinkel *et al*<sup>19</sup> using human spleen substrate found 4.1% ANA positivity and observed that this was related to recurrent foetal wastage. Farnam *et al*<sup>13</sup>, in his study of 219 pregnant women found an ANA positivity of 10% at titres of >1:80. They suggested that the frequency of ANA increased with increasing parity but did not find any significant difference of its occurrence among the trimesters. He concluded that it was not necessarily associated with perinatal abnormalities.

It is apparent from the above that there are wide disparities in the results obtained by various investigators. These could be attributed to their use of different substrates in their test systems or that the cut-off point regarded as positivity varied.

Our findings showed that only 1.4% of 146 normal healthy individuals had positive ANA at a dilution of 1:20 compared to none among the controls. The frequency with which autoantibody occur in pregnancy is not significantly different from that of a group of non-pregnant healthy women. This was much lower than that observed by other investigators. The positive cases were not found to have any clinical manifestations of autoimmune disease. None experienced any complications throughout pregnancy. All had normal live deliveries.

A more significant figure would be obtained with an increase in sample size. Thus, larger studies of our local community may be needed to determine whether there actually is a correlation. An improved longitudinal study involving testing serum samples from each individual at every stage of pregnancy and post-partum is needed. Additional serological autoimmune screening involving autoantibodies like anti-SSA/Ro, anti-SSB/La and anti-phospholipid antibodies need to be considered. The women who have been shown to be positive for one or more autoantibodies detected will be followed-up to detect any changes both clinically as well as serologically.

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