



## CO-RELATION OF TPO AND CD19-B cells WITH RECURRENT SPONTANEOUS ABORTION (RSA)

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**BACKGROUND:** Recurrent spontaneous abortion (RSA) is defined as three or more consecutive pregnancy losses prior to the 20th week of gestation. Around 15% of clinical pregnancies end by spontaneous abortion. The etiology of recurrent spontaneous abortion is often unclear and may be multi-factorial, with much controversy regarding diagnosis and treatment.

However, the majority of cases of RSA remains unexplained and is found to be associated with certain autoimmune antibodies that may play major role in the immunologic failure of pregnancy and may lead to abortion.

Several mechanisms have been suggested to explain the role of thyroid auto-antibodies in recurrent miscarriages. Thyroid auto-antibodies affect the maternal thyroid gland. The presence of thyroid auto-antibodies in women with normal thyroid function could be associated with a subtle deficiency in the availability of thyroid hormones (a fall in circulating free thyroid hormones within the reference range) or a lower capacity of the thyroid gland to adequately rise to the demand for augmented synthesis of thyroid hormones required in pregnancy.

Previous research says that the proportion and number of B cells (cd19), were significantly increased in the first trimester of pregnancy in RSA women compared with normal pregnant control. A significant increased number of circulating cd56+ NK cells was found in RSA women who miscarried compared with RSA women who delivered.

**OBJECTIVE:** The purpose of this research is to study the association of TPO and B-cells (cd19) with recurrent spontaneous abortion and to study the effect and outcome of the drug.

**STUDY DESIGN:** We conducted a retrospective cohort study of RSA patients from department of Reproductive Immunology, Shanghai First Maternity and Infant Hospital, Shanghai, China. From Jan 2017 - June 2017 there were total of 4020. This study includes 2106 patients. Out of which 1748 patients were RSA patients (case group) and 358 patients were of one-time miscarriage patients (control group), who were eligible for the analysis. However, the rest 1914 patients were RSA patients with normal lymphocytes and CD19 marker, which were not eligible for the study. P value of < 0.05 was considered significant.

P value of  $> 0.05$  was not considered to be statistically significant.

Association among variables of case group and control group were assessed using Pearson Chi square test. As all the parameters don't obey normal distribution therefore non-parametric statistical analysis was done.

**RESULT:** In the retrospective cohort study. Analysis of TPO and cd19 shows significant difference  $P=0.00$ . Co-relation of TPO and cd19 before and after treatment shows no significant difference. Treatment with drug group shows no significant difference. Aspirin use by RSA patients with abnormal TPO shows no significant difference.

**CONCLUSION:** Co-relation of TPO and cd19 of RSA patients shows no significant difference. Separate analysis of TPO and cd19 of RSA shows significance difference. Which concludes that the combination of TPO and cd19 is not a factor of miscarriage, therefore the separate analysis of TPO and cd19 is a factor of miscarriage.

**Key words:** RSA, TPO, CD19 B-cells.

## BACKGROUND

Recurrent spontaneous abortion (RSA) is defined as three or more consecutive pregnancy losses prior to the 20th week of gestation. Around 15% of clinical pregnancies end by spontaneous abortion. It is estimated that almost every second woman will have at least one miscarriage during her reproductive years. Recurrent spontaneous abortion (RSA) is reported for around 1–5% of couples. The figures vary, depending on the diagnostic criteria used to define RSA; while the WHO definition stipulates three or more consecutive miscarriages before the end of the 20th completed week of gestation, the American guidelines define RSA as two failed intrauterine pregnancies. Recurrent spontaneous abortion has a multi-factorial pathogenesis which often remains unexplained, and it represents a clinical challenge, both for the affected women and their treating physicians [1].

The etiology of recurrent spontaneous abortion is often unclear and may be multi-factorial, with much controversy regarding diagnosis and treatment. Reasonably accepted etiologic causes include, genetics, anatomical, endocrine, placental anomalies, hormonal problems, infection, smoking and alcohol consumption, exposure to environmental factors, psychological trauma and stressful life event, certain coagulation, and immune-regulatory protein defects.

Thyroid Autoimmunity (TAI) is the most common autoimmune disorder in women of reproductive age with a prevalence varying between 5 and 15%. It is five- to ten-times more common in women than in men and can be present without thyroid dysfunction, thus remaining undiagnosed. The effects of oestrogens in promoting autoimmunity, genetic factors and perhaps maternal microchimerism and chromosome X abnormalities, may potentially explain the high prevalence of TAI in women. Pregnancy is associated with profound changes in thyroid function [2].

## **Tpo and miscarriage:**

Spontaneous pregnancy loss is a surprisingly common occurrence, with approximately 15% of all clinically recognized pregnancies resulting in pregnancy failure. Spontaneous pregnancy loss can be physically and emotionally taxing for couples, especially when faced with recurrent losses [3].

A significant association between thyroid autoimmunity and risk of reproductive failures, such as miscarriage and infertility has been shown by various studies. Studies on TAI and recurrent spontaneous abortion are somewhat mixed, although the majority of studies have shown an association [4].

The heterogeneity of immunological risk factors shown in the study indicates the usefulness of detecting alloimmune factors as well as auto-antibodies in patients' recurrent miscarriage of unknown etiology. This may be helpful to analyze the therapeutical effectivity of various treatment in better characterized group of patients and to explain unsuccessful results of treatment in patients with recurrent miscarriage of unknown etiology [5].

Anti-thyroid auto-antibodies are auto-antibodies targeted against one or more components on the thyroid. The most clinically relevant anti-thyroid auto-antibodies are anti-thyroid peroxidase antibodies (anti-TPO antibodies), thyrotropin receptor antibodies (TRAbs) and thyroglobulin antibodies. TRAbs are subdivided into activating, blocking and neutral antibodies, depending on their effect on the TSH receptor. Anti-sodium/Iodide (Anti-Na<sup>+</sup>/I<sup>-</sup>) symporter antibodies are a more recent discovery and their clinical relevance is still unknown. Graves' disease and Hashimoto's thyroiditis are commonly associated with the presence of anti-thyroid auto-antibodies. Although there is overlap, anti-TPO antibodies are most commonly associated with Hashimoto's thyroiditis and activating TRAbs are most commonly associated with Graves' disease. Thyroid microsomal antibodies were a group of anti-thyroid antibodies; they were renamed after the identification of their target antigen (TPO) [6].

According to a publication of the American Thyroid Association in October 2016, auto-immune thyroid disease is very common in women of childbearing age and can lead to either an overactive (Graves' disease, hyperthyroidism) or underactive thyroid (Hashimoto's Thyroiditis, hypothyroidism). Auto-immune thyroid disease occurs when the body makes antibodies that attack the thyroid and turn it on or off. This is characterized by positive TPO and/or thyroglobulin antibodies and is most commonly associated with increased risk of developing hypothyroidism. Women with positive TPO antibodies have been shown to have an increased risk of pregnancy complications, including miscarriage and preterm labor. Autoimmune thyroid disease has been shown to be more common in women seeking treatment for infertility. This study sought to determine the effect of autoimmune thyroid disease on the success of assisted reproduction techniques (infertility treatments), specifically in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). [7] They concluded that, women with auto-immune thyroid disease and normal thyroid function have less successful pregnancy outcomes (fewer live births and more likely to have a miscarriage) following fertility treatments (IVF or ICSI) than women without autoimmune thyroid disease. The underlying mechanism is not

known but future studies should be designed to better understand this process and hopefully lead to identification of appropriate prevention strategies [7].

According to a study in Chandigarh, India in the year 2013, the prevalence of thyroid autoimmunity was higher in pregnant women with a history of recurrent abortion compared with the healthy pregnant control population. Even though the TSH value was higher in the TPOAbc group than in the TPOAbK group, the prevalence of subclinical hypothyroidism was comparable between the two groups. There was no absolute difference in the prevalence of miscarriage between subclinical hypothyroid and euthyroid pregnant women irrespective of TPO status. However, the odds of having miscarriage in the TPOAbC group increases with rising TSH level compared with normal TSH level [8].

According to China medical university, Shenyang, finding showed that isolated TAI with normal TSH level was associated with a higher risk of miscarriage, but when both TAI and SCH (subclinical hypothyroidism) coexisted in an individual, the risk of miscarriage increased significantly. This situation was more remarkable with increasing serum TSH levels [9].

### **CD19 B-cell and miscarriage:**

Approximately, 20% of women with RPL have autoimmune abnormalities. In women with RPL, the presence of autoimmunity is often associated with cellular immune abnormalities, such as increased NK cell levels and TH1/TH2 cell ratios [10].

B-lymphocyte antigen CD19 is also known as CD19 Molecule (Cluster of Differentiation 19), B-Lymphocyte Surface Antigen B4, T-Cell Surface Antigen Leu-12 and CVID3 is a trans-membrane protein that in humans is encoded by the gene CD19. [5][6] In humans, CD19 is expressed in all B lineage cells, except for plasma cells, and in follicular dendritic cells. CD19 plays two major roles in human B cells. It acts as an adaptor protein to recruit cytoplasmic signaling proteins to the membrane and it works within the CD19/CD21 complex to decrease the threshold for B cell receptor signaling pathways. Due to its presence on all B cells, it is a biomarker for B lymphocyte development [11].

Through study of CD19 transgenic and knockout mouse models, it becomes clear that CD19 plays a critical role in maintaining the balance between humoral, antigen-induced response and tolerance induction [12].

According to the university at Magdeburg Germany, in the year 2013, they observed significantly augmented percentages of CD19+CD24hiCD27+ Breg in normal pregnant when compared to non-pregnant women. Notably, women suffering from miscarriages presented significantly lower percentages of CD19+CD24hiCD27+ Breg than women having normal pregnancies in the first trimester. The levels observed in these patients were comparable with those measured in non-pregnant women. Thus, their data indicate that factors present in normal pregnancies but not in failing pregnancies stimulate the expansion of regulatory B cells [13].

According to the Health and Environment, University Hospital, Linkoping, Sweden, in the year 2001, during normal pregnancy, the absolute leukocyte number rises, because of increased numbers of granulocytes,

with unchanged numbers of lymphocytes and monocytes. It was found that the proportion and number of B cells (CD19+), were significantly increased in the first trimester of pregnancy in RSA women compared with normal pregnant controls, in agreement with the result [14].

Alterations in normal balance of B cell subsets have been reported in various rheumatic diseases. In this study, we report a woman with a history of recurrent pregnancy losses (RPL) and infertility who had low levels of memory B cells. A 35-year-old woman with a history of RPL and infertility was demonstrated to have increased peripheral blood CD19+ B cells with persistently low levels of memory B cell subsets.

Prior to the frozen donor egg transfer cycle, prednisone and intravenous immunoglobulin G (IVIg) treatment was initiated and patient achieved dichorionic diamniotic twin pregnancies. During pregnancy, proportion (%) of switched memory B cells CD27+IgD- increased, while percent of total CD19+ B cells and CD27-IgD+ naive B cells were gradually decreased with a high dose IVIg treatment. She developed cervical incompetence at 20 weeks of gestation, received a Cesarean section at 32 weeks of gestation due to pre-term labor, and delivered twin babies. B cell subset abnormalities may be associated with infertility, RPL and preterm labor, and further investigation was needed. [15]

The proportion and number of B cells (CD19+), were significantly increased in the first trimester of pregnancy in RSA women compared with normal pregnant controls. [14]

In a study it was examined the frequency and regulatory function of cd5+, cd19+B10 cells in an AD mouse model. The result showed that the percentage of cd5+ cd19+ B10 cells increased while the frequency of IL-10- producing B cells in CD19+B cells decreased in the mice of AD group. [16]

Previous research says that the proportion and number of B cells (cd19) were significantly increased in the first trimester of pregnancy in RSA women compared with normal pregnant control. A significant increased number of circulating cd56+ NK cells was found in RSA women who miscarried compared with RSA women who delivered. [17]

## MATERIALS AND METHODS

### Study population:

From Jan 2017- June 2017 there were total of 4020 patients in this department. This study includes 2106 patients. Out of which 1748 patients were RSA patients (case group) and 358 patients were of one-time miscarriage patients (control group), who were eligible for the analysis.

### Eligibility criteria:

All the pregnant and non-pregnant women with history of two or more consecutive miscarriages (RSA), All the pregnant and non- pregnant women with history of one-time miscarriage, All the RSA patients going through IVF and IUI All the RSA patients with implantation failure and biochemical pregnancy and All the RSA patients with diabetes, hypertension and PCOD

### Exclusion criteria:

All the women with known autoimmune disorder already on treatment for thyroid dysfunction, History of cervical incompetence or any other uterine pathology and Chromosomal abnormalities

### **Method and design:**

In this retrospective cohort study. The medical records of women with a history of RSA, who registered in Shanghai first maternity and infant care hospital, china, between January 2017 to June 2017 were collected, reviewed, and analyzed.

The data of TPO, and lymphocytes CD 19 markers of the blood tested on the same date was recorded. In the patient's history who had the abnormal data of either TPO, or CD markers for the first time, was recorded in the Excel as before treatment group and with normal and abnormal data of TPO, and CD markers were recorded as after treatment group.

### **Statistical analysis:**

For the entry of statistical data, the computer package used was Microsoft Excel. The accumulated data were analyzed with Statistical Package for Social Science (SPSS) of Window version 22. P value of  $< 0.05$  was considered significant. P value of  $> 0.05$  was not considered to be statistically significant. Mean of parameters in case and control group were expressed as mean  $\pm$  standard deviation (mean  $\pm$ SD). Association among variables of case group and control group were assessed using Pearson Chi square test. Independent t-test was used to compare the variables of two groups, and 95% Confidence Interval (CI), as appropriate.

## **RESULTS**

Systematic review and meta-analysis of the study showed the significant association of thyroid autoantibody and CD19 B-Cells as a factor of RSA.

On the basis of definition that TPO and CD19 B-cells is represented by normal value of TPO = 060, CD19= 4.7-19.3.

On separately comparing normal and abnormal cd19 of case and control Group, probability shows  $< 0.05$ . Therefore, we can conclude that Cd19 could be associated with RSA (Table 5). On comparing normal and abnormal TPO with case and control group, probability shows  $< 0.05$ . Therefore, TPO shows an association with RSA (Table 6)

In the cross tabulation in (Table 7) and (Table 8) on combining TPO and cd19 by forming groups and comparing with successful pregnancies,  $P = 1.738$  shows no significant difference.

Under different treatment, different group of drugs have different pregnancy success rate, out of 2099 patients, 144 patients got pregnant and 1955 did not get pregnant till date. ( $P = 0.535$ ) which is not statistically significant (Table 9)

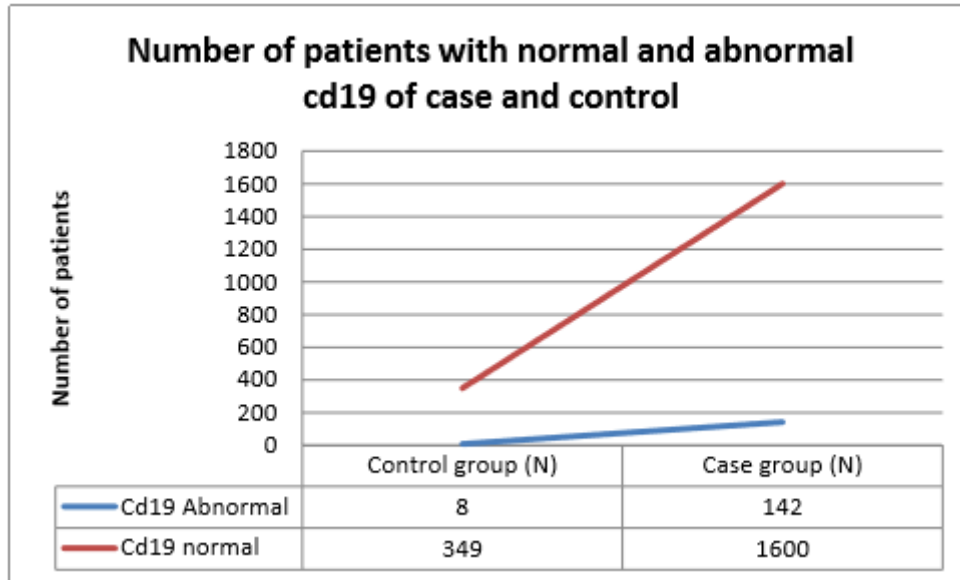
Use of Aspirin drug shows no significance with normal and abnormal TPO patients ( $P = 0.640$ ) (table 12)

1.1 SEPARATE ANALYSIS OF TPO AND B-cell CD19

Parameter	Control group (N)	Case group (N)
Cd19 Abnormal	8	142
Cd19 normal	349	1600

Normal CD19 =4.7-19.3; abnormal CD19= high or low; RSA= recurrent spontaneous

**Table 1:** Comparison of case and control group of RSA patients who had normal and abnormal CD 19 markers.



**Graph 1:** Comparison of case and control group of RSA patients who had normal and abnormal CD 19 markers.

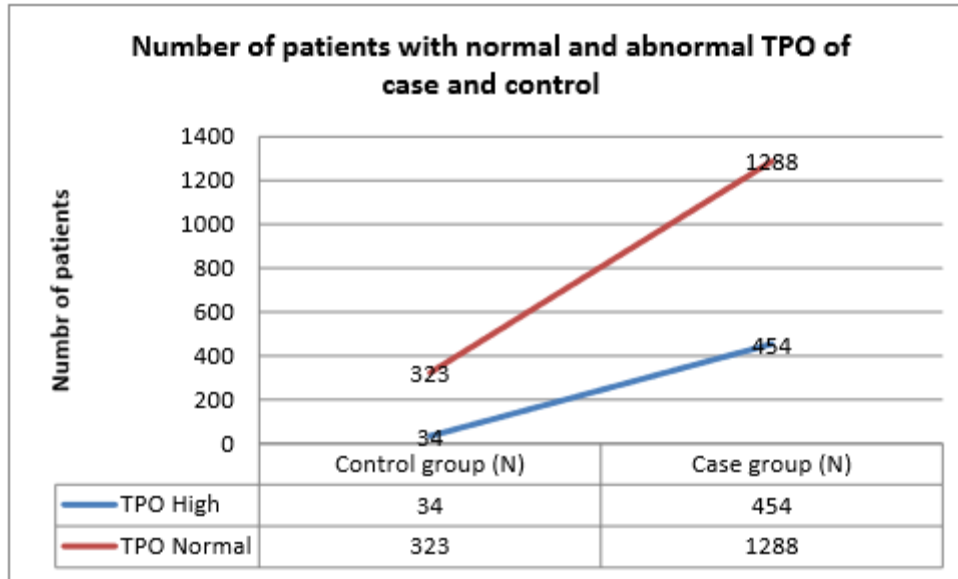
1. The blue line in the graph indicates patients with abnormal CD19 are very less in case and control group whereas red line indicated patients with normal cd19 are higher in case and control group of RSA patients.
2. On comparing normal and abnormal cd19 of case and control Group, probability (P=0.00) shows <0.05 is statistically significant

Parameter	Control group (N)	Case group (N)
TPO High	34	454
TPO Normal	323	1288

TPOantibody = Thyroid peroxidase antibody; Normal TPO=0-60; abnormal TPO= >60

RSA= recurrent spontaneous

**Table 2:** Comparison of case and control group of RSA patients who had normal and abnormal TPO markers.



**Graph 2:** Comparison of case and control group of RSA patients who had normal and abnormal TPO markers

1. The blue line in the graph indicates, patients high TPO are very less in case and control group whereas red line indicated patients with normal TPO are higher in case and control group, of RSA patients
2. On comparing normal and abnormal TPO with case and control group, probability shows < 0.05 (P=0.00) showing statistically significance.

**1.2 CO-RELATION OF TPO AND CD19 IN RSA PATIENTS**

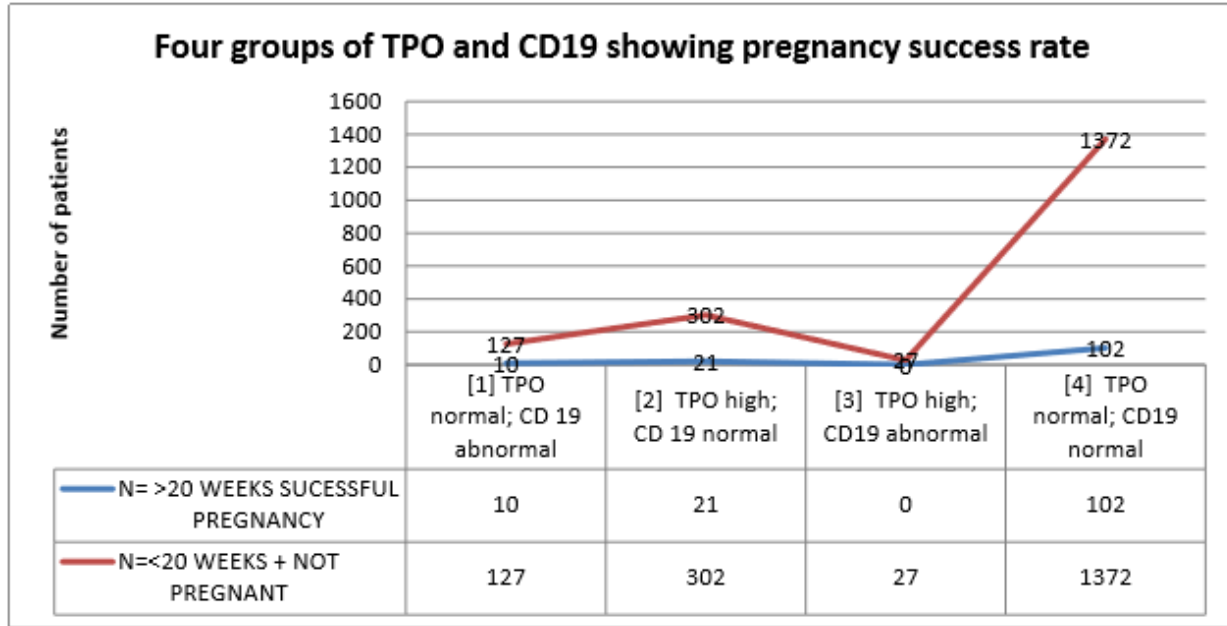
**Before treatment:**

Parameters grouped	N= >20 WEEKS SUCCESSFUL PREGNANCY	N=<20 WEEKS + NOT PREGNANT
[1] TPO normal; CD 19 abnormal	10	127
[2] TPO high; CD 19 normal	21	302
[3] TPO high; CD19 abnormal	0	27
[4] TPO normal; CD19 normal	102	1372

N= Number of patients; TPO antibody = Thyroid peroxidase antibody; RSA= recurrent spontaneous

**Table 3:** Comparison of (combined and grouped) TPO and CD19 markers of RSA patients who had successful pregnancies with >20 weeks and who had <20 weeks gestation with no successful pregnancy.





**Graph 3:** Comparison of (combined and grouped) TPO and CD19 markers of RSA patients who had successful pregnancies with > 20 weeks and who had <20 weeks gestation with no successful pregnancy

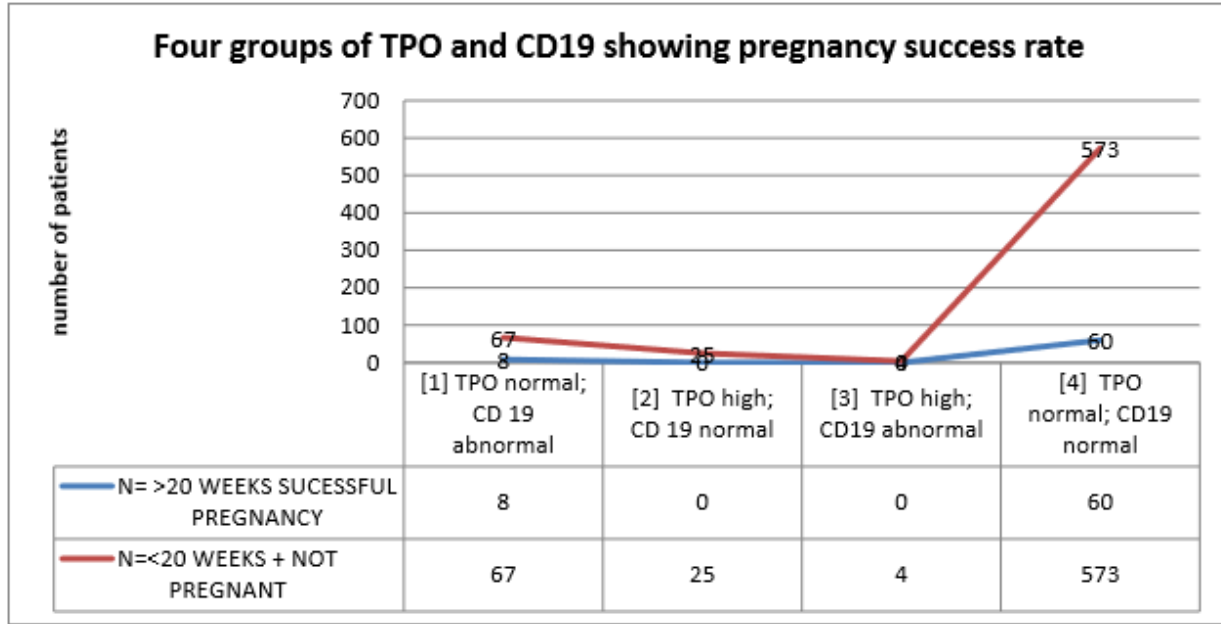
1. There are 4 groups formed after combining TPO and CD19. Group 1- TPO normal + CD19 abnormal; Group 2- TPO high + CD19 normal; group 3- TPO high CD19 abnormal; group 4- TPO- Normal + CD19 normal.
2. Red line indicating unsuccessful pregnancies and blue line indicating successful pregnancies. On combining TPO and CD19 by forming groups and comparing with successful pregnancies, we can see that, larger number of patients after treatment lie in normal TPO and normal CD19 group
3. Probability > 0.05 with 1.913 shows no significant difference.
4. The co-relation of TPO and cd19 does not show association with RSA.

**After treatment:**

Parameters grouped	N= >20 WEEKS SUCCESSFUL PREGNANCY	N=<20 WEEKS + NOT PREGNANT
[1] TPO normal; CD 19 abnormal	8	67
[2] TPO high; CD 19 normal	0	25
[3] TPO high; CD19 abnormal	0	4
[4] TPO normal; CD19 normal	60	573

N= Number of Patients; TPO antibody = Thyroid peroxidase antibody; RSA= recurrent spontaneous

**Table 4:** Comparison of (combined and grouped) TPO and CD19 markers of RSA patients who had successful pregnancies with > 20 weeks and who had <20 weeks gestation with no successful pregnancy.



**Graph 4:** Comparison of (combined and grouped) TPO and CD19 markers of RSA patients who had successful pregnancies with > 20 weeks and who had <20 weeks gestation with no successful pregnancy.

1. There are 4 groups formed after combining TPO and CD19, Red line indicating unsuccessful pregnancies and blue line indicating successful pregnancies. On combining TPO and CD19 by forming groups and comparing with successful pregnancies, we can see that larger number of patients after treatment lie in normal TPO and normal cd19 group.
2. Probability > 0.05 with 1.913 shows no significant difference.
3. The co-relation of TPO and CD19 therefore does not show association with RSA.

**1.3 TREATMENT AND PREGNANCY SUCCESS RATE**

Drug groups	N= >20 weeks pregnant	N=<20 weeks preg +not pregnant
A	7	95
A, Pro, H	20	344
A, Pro, P	49	557
A, M, H	41	533
P, H, M	27	426
Total	144	1955

**Legends**

A=Aspirin

Pro=Progesterone

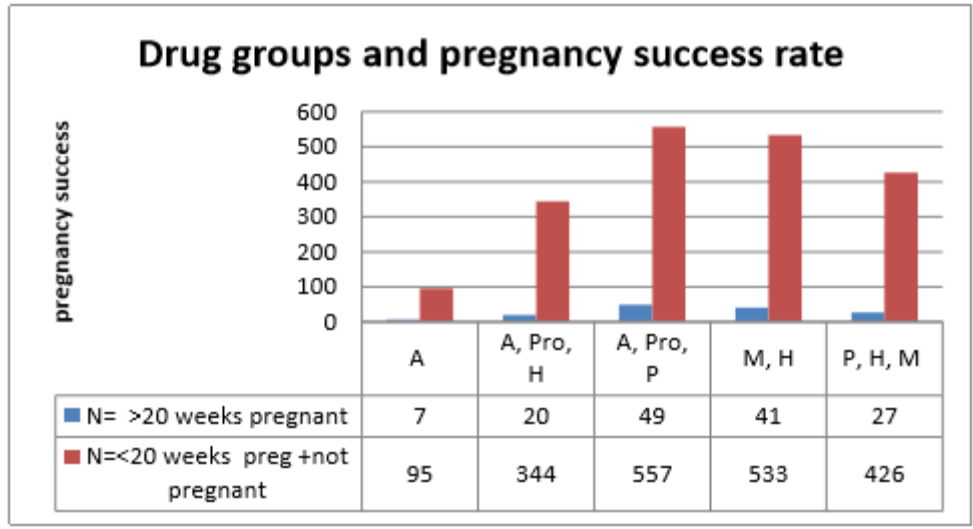
P=Prednisone

H=Heparin

M=Metformin

N=number of patients; P=0.536

**Table 5:** Different group of drugs used in RSA patients and their pregnancy success rate



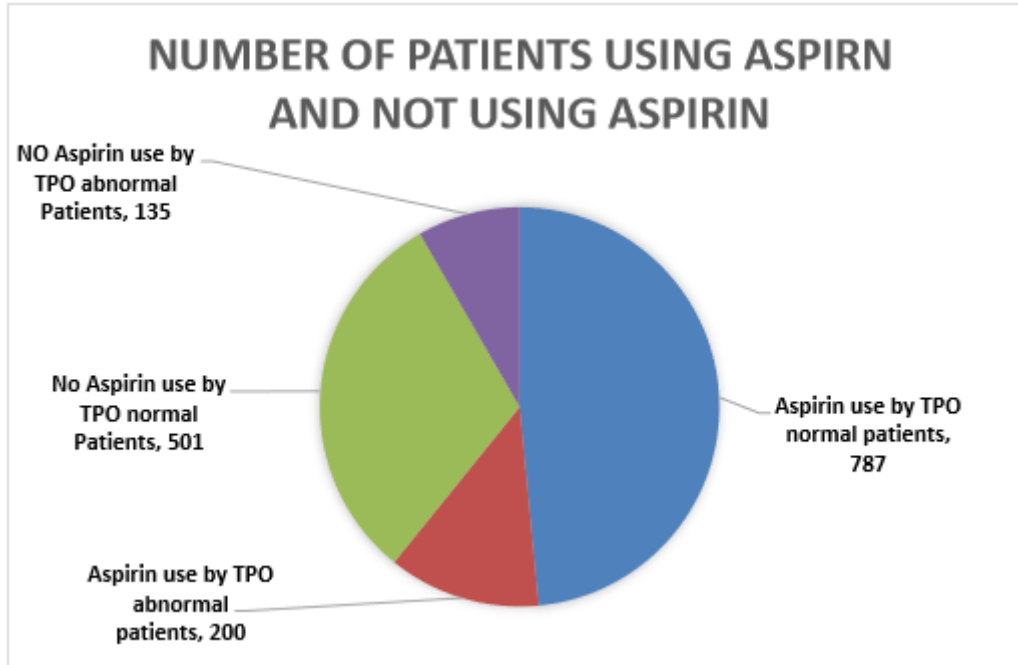
P=0.536

**Graph 5:** Different group of drugs used in RSA patients and their pregnancy success rate

1. The red color bar showing unsuccessful pregnancy above 500 cases in RSA patients, and blue color bar showing successful pregnancy less than 100 cases in RSA patients with abnormal TPO and lymphocytes CD markers.
2.  $P > 0.05$  with 0.536 showing no significance. Therefore, conclusion can be made that these group of treatment does not show a valuable effect on RSA patients
3. In most of the drug groups Aspirin is used, therefore aspirin use is high in RSA patients. Drug group of aspirin, progesterone and prednisone is higher than any other group. Second drug group to be used most is Metformin and Heparin.
4. Patients under Aspirin and Heparin Medications are more than any other medication. Successful Pregnancy is very low with the undergoing treatment.

DRUG	TPO Normal	TPO Abnormal
Aspirin (yes)	787	200
Aspirin (no)	501	135

**Table 6:** Aspirin use and no aspirin use by TPO normal and abnormal patients.



**Graph 6:** Aspirin use and no aspirin use by TPO normal and abnormal patients

1. In the pie chart we can see the number of TPO patients are high with aspirin use compared to no aspirin using patients, although analysis showing no significant difference ( $P=0.640$ )

## DISCUSSION

Several mechanisms have been suggested to explain the role of thyroid auto-antibodies in recurrent miscarriages. Firstly, thyroid auto-antibodies affect the maternal thyroid gland. The presence of thyroid auto-antibodies in women with normal thyroid function could be associated with a subtle deficiency in the availability of thyroid hormones (a fall in circulating free thyroid hormones within the reference range) or a lower capacity of the thyroid gland to adequately rise to the demand for augmented synthesis of thyroid hormones required in pregnancy. Secondly, thyroid auto-antibodies may cross the placenta and can affect the fetus where 40% of neonates born to mothers with elevated TPO Ab titres at birth. They might be an indicator of an underlying enhanced global autoimmune state. This itself can have a direct adverse effect on placental or fetal development. They can affect the maternal gestational tissues like anti-phospholipid antibodies, which cause decidual vascularity and placental insufficiency, Thirdly, the thyroid auto-antibodies may be the result rather than the cause of recurrent pregnancy loss. This can happen as a consequence of early immune interaction resulting in rejection of the fetus [18].

Fifth antithyroid antibodies reflect generalized activation of the immune system. StagnaroGreen et al suggested that the auto-antibodies are secondary markers of autoimmune risk rather than the specific causative factors. However, the role of thyroid autoantibody positivity as a causative factor of abortion still needs more explanation [19]

The University of Calcutta, India, in the year 2009. Based on the result of this study, evaluation of APLA status is now recommended as a routine unit policy on investigation for recurrent miscarriages, as >1 in 4 women who met the criteria were noted to be antibody positive. APLA is now included as a routine unit policy of 'investigations directed towards recurrent miscarriage and late pregnancy loss' in the study hospital [19]. Therefore, it should also be suggested to make TPO as a routine unit policy on investigation for recurrent miscarriage patients.

Previous research says that the proportion and number of B cells (cd19), were significantly increased in the first trimester of pregnancy in RSA women compared with normal pregnant control. A significant increased number of circulating cd56+ NK cells was found in RSA women who miscarried compared with RSA women who delivered. [20]

Studies have shown cd19 B-cells were increased in pregnancy in RSA women. One study has shown low level of cd19 B-cells in women suffering from miscarriage.

In this retrospective cohort study separate analysis of TPO and CD19 of case and control group shows a significant difference, which is consistent with other research.

In this retrospective cohort study by combining and grouping TPO and cd19 to see the corelation no significant difference was found.

TPO has  $P=0.166$  and CD19 has  $P=0.308$ , which is not statistically significant. It signifies that the combination of cd19 and TPO is not a factor of RSA.

## CONCLUSION

Thyroid auto-antibody are relatively common in women of reproductive age. Thyroid autoimmunity is associated with adverse pregnancy outcome as miscarriage. The analysis of TPO and CD19 B-cells of case and control group shows significant difference in this study, which is consistent with other studies. The combined co- Relation of TPO and CD19 B-cells show no significance as a factor of RSA, which suggests that TPO and cd19 B-cells has no association with each other in causing miscarriage. The group of drug used by most of the RSA patients showed no significant difference. Aspirin use by miscarriage patients with abnormal TPO is high therefore showing no significant difference.

### Limitations of the study:

The reason behind these results and the limitations of the present study could be. Most of the RSA patients were going through IVF and IUI. Most of them were suffering from hypertension, diabetes and PCOD, as large number of patients were suffering from these diseases therefore it was not possible to exclude them from the study. The sample size was small.

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