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Original Research Article

Study the immunomodulatory effect of hydroxychloroquine in recurrent pregnancy loss patients in their current pregnancy

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ABSTRACT

Background: Recurrent pregnancy loss is defined as 3 or more pregnancy loss consecutively. in most of the cases cause of pregnancy loss remains unclear. lethal chromosomal abnormality is commonly found in product of conception. HCQ has an impact on innate immunity by decreasing the circulatory level of interleukin-1, TNF-alpha and interferon-gamma. In this way this creates a normal environment that favour normal pregnancy development. This study was aimed to find out the effect of HCQ in RPL patients who have raised TNF alpha and anti-nuclear antibody.
Methods: This is a clinical interventional cross over design study. All patients included in the study were given 200 mg HCQ twice a day with ecosprin 75 mg HS and folic acid 5 mg. After first trimester iron and calcium are also included in the treatment along with previous treatment. HCQ is continued till delivery.
Results: The result of this study shows that there is a significant decrease in the serum value of TNF alpha and after treatment with HCQ in three months. The p value is less than 0.001 for TNF alpha and 0.001 for ANA. This shows that HCQ treatment in recurrent miscarriage patients decreases the serum value of TNF and ANA to a significant level and helps in continuation of pregnancy.
Conclusion: It can be concluded that the use of HCQ showed a decrease in TNF alpha and antinuclear antibody in an ongoing pregnancy, which diminished the chances of recurrent pregnancy loss.

Keywords: HCQ, Immunomodulatory effect, Recurrent pregnancy loss

INTRODUCTION

Recurrent pregnancy loss is a common problem in reproductive age group females. this causes physical and emotional stress to couple. To date no definite treatment plan is present for these patients. Recurrent pregnancy loss is defined as 3 or more pregnancy loss consecutively. in most of the cases cause of pregnancy loss remains unclear. lethal chromosomal abnormality is commonly found in product of conception. The incidence of sporadic miscarriage is 10 to 15%. foetal development is usually stopped before 10 weeks of pregnancy.[1] Chances of normal embryonic karyotype are increased after third pregnancy loss.[2] Investigations present at present fails to find out the cause in ~50% of cases. Based on several hypotheses thrombosis, endothelial injury and immune dysfunction could be the cause in the unexplained pregnancy loss patients. Few animal model studies have demonstrated that haemostatic system may be responsible for implantation and placental development.[3,4] In women with history of recurrent pregnancy loss, a prothrombotic condition is noticed outside of pregnancy and without known thrombophilia.[5-7] This pro-thrombotic state could reflect chronic endothelial damage in these patients.[8,9] Empirical treatment with low dose ecosprin with or without low molecular weight heparin did not prevent

pregnancy loss in all patients.[10-13] Continuation of semi allogenic foetus in normal pregnancy is dependent on maternal immune tolerance. It is reported in many studies that there is dysregulation of immune system in women with RPL.[14-16] Th-2 and T-reg preponderance in normal pregnancy shifts to Th-1 and Th-17 in RPL patients.[17,18] Immunomodulatory treatment in the form of paternal leucocyte immunisation, intravenous immunoglobulins and small doses of corticosteroids have been used in these patients without any definite results.[19-21]

Chloroquine and hydroxychloroquine (HCQ) is the drugs for malaria treatment. HCQ has an impact on innate immunity by decreasing the circulatory level of interleukin-1, TNF-alpha (tumour necrosis factor alpha) and interferon-gamma.[22-24] In this way this creates a normal environment that favour normal pregnancy development decreases APL plasma levels and interfere with both endothelial and cell activation and TNF alpha production. These are the 2 major pathways responsible for APS (antiphospholipid syndrome).[25-28]

HCQ has an extensive safety profile in pregnancy and lactation. This study was aimed to find out the effect of HCQ in RPL patients who have raised TNF alpha and anti-nuclear antibody.

The objective was to find out the effect of HCQ on the serum level of TNF alpha and antinuclear antibody in recurrent miscarriage patients in their current pregnancy and pregnancy outcome.

METHODS

Study design

This was a clinical interventional cross-over study.

Study place

The study was done at Sparsh Hospital Nasrapur Tirwa Road Kannauj, UP, India.

Study duration

The duration of study was from May 2022 to March 2024.

Sample size

60 pregnant patients in their early first trimester (5 to 10 weeks) with a history of previous 2 or more miscarriages in first trimester were included in the study.

Inclusion criteria

Patients with a history of 2 or more previous miscarriages in the first trimester of pregnancy, aged between 21 to 35 years; patients with normal karyotype of both the patients, women with informed consent, patients who took treatment in their previous pregnancy other than HCQ were included.

Exclusion criteria

Patients with any congenital uterine anomaly, patients with uterine fibroid and adenomyosis, patients with known contraindication to a treatment by HCQ, and patients with previous long-term exposure to HCQ were excluded.

Intervention

All patients included in the study were given 200 mg HCQ twice a day with ecosprin 75 mg HS and folic acid 5 mg. After first trimester iron and calcium are also included in the treatment along with previous treatment. HCQ was continued till delivery.

Study plan and procedure

All selected patients were advised serum TNF alpha and anti-nuclear antibody on their first visit. After that HCQ is started. Patients are followed up after 15 days to know any side effects of the drug. LFT, KFT and CBC are advised to check renal and liver function. Patients also asked about visual and neurological impairment. Any complain of skin rash and hypersensitivity. Then follow up every month was done. This investigation is advised at each visit. after 3 months serum TNF alpha and anti-nuclear antibody was done. Patients follow up till delivery and after that at 3 months.

Statistical analysis

Qualitative variables have been presented as frequency and percentage. quantitative variables first observed for the normality of distribution using Q-Q plot. if data is normally distributed, it has been presented as mean and standard deviation. the data will be presented as median and interquartile range if it is not normally distributed. since in inferential statics we are doing before and after comparison, we would be using paired t-test or Wilcoxon rank test.

RESULTS

Patients included in this study tolerated HCQ very well. Some patients complain of digestion upset or headaches at the start of treatment. These problems are managed symptomatically. One patient was lost in the follow-up. The majority of pregnancies resulted in the birth of a healthy baby. There is a relatively equal distribution of male and female babies. Four women had IUD at different gestational weeks (Table 1). The mean TNF level is relatively high (68.7), suggesting potential inflammation or immune system activity. A significant number of samples were missing for ANA (41 out of 59). The mean ANA level is also elevated (30.8), indicating possible autoimmune activity (Table 2).

4 patients had a loss of pregnancy at 24, 26, 9 and 8 weeks of pregnancy. 2 patients stopped HCQ. 21 patients were delivered by LSCS and 34 by vaginal delivery. 4 patients delivered between 34 to 36 weeks; rest of the patients were delivered after 37 weeks. LSCS done for obstetric reasons.

Table 1: Outcome of pregnancy.

Outcome of pregnancy	Count	% total	Cumulative %
IUD at 24 weeks	1	1.7	1.7
Abortion at 8 weeks	1	1.7	3.4
Abortion at 9 weeks	1	1.7	5.1
IUD at 26 weeks	1	1.7	6.8
Female baby	26	44.1	50.8
Male baby	29	49.2	100

Table 2: Descriptive analysis of serum value of TNF alpha, anti-nuclear antibody and serum homocysteine.

Descriptives	N	Missing	Mean	Median	SD	IQR	Minimum	Maximum
TNF	59	0	68.7	33.2	95.7	46.8	8.21	488
ANA	18	41	30.8	24.8	17.9	14.3	8.33	75

Table 3: Demographic of the delivered babies.

Gender	Count	% total	Cumulative %
Female baby	26	44.1	50.8
Male baby	29	49.2	100

Table 4: Paired sample t test value.

Paired sample t test	Statistics	P value
TNF	1484	<0.001
ANA	105	0.001

One baby had a cleft lip and palate, which was repaired and the baby is doing well. The weight of the babies was normal for their gestational age.

55 babies were delivered. There is a relatively equal distribution of male and female babies. 26 female babies were born, representing 44.1% of the total. 29 male babies were born, representing 49.2% of the total (Table 3).

The mean for TNF level was 21.08 whereas that of the ANA level was at 11.09. The median and SD of TNF at start was 11.00 and 39.12 respectively as opposed to 10.50 and 3.80 for ANA. The mean TNF level decreased significantly after 3 months (from 68.7 to 21.08), suggesting a reduction in inflammation or immune activity. The mean ANA level also decreased (from 30.8 to 11.09), indicating a potential improvement in autoimmune status (Figure 1-4).

There is a significant difference between the TNF levels before and after the intervention. The p-value is very small, indicating strong evidence against the null hypothesis (no difference). Similar to TNF, there is a significant difference between the ANA levels before and after the intervention. The p value is again very small, suggesting a strong effect (table 4).

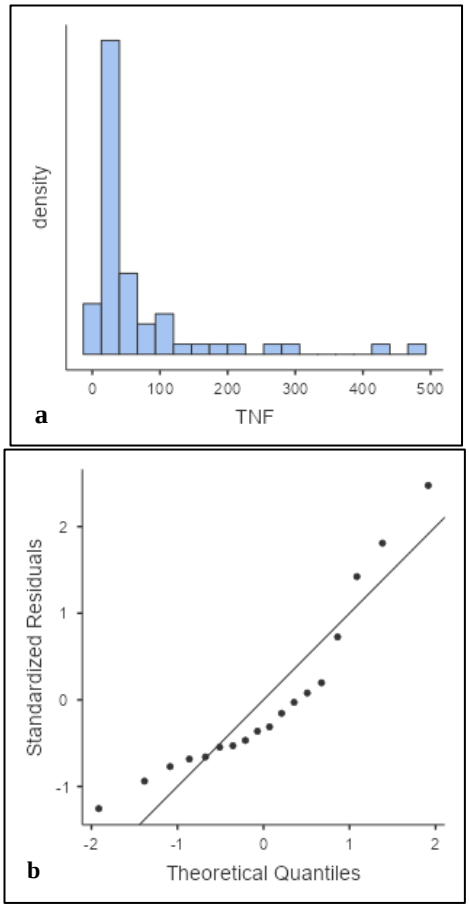

Figure 1 (a and b): Descriptive analysis of serum value of TNF at start.

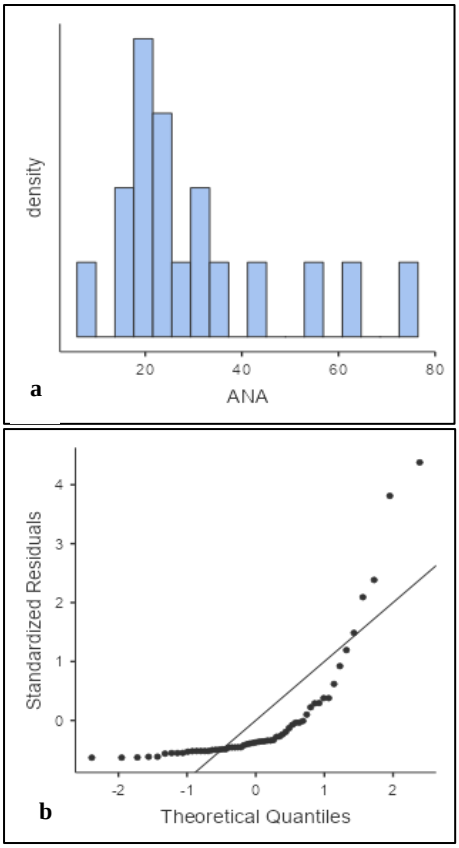

Figure 2 (a and b): Descriptive analysis of serum value of ANA level at start.

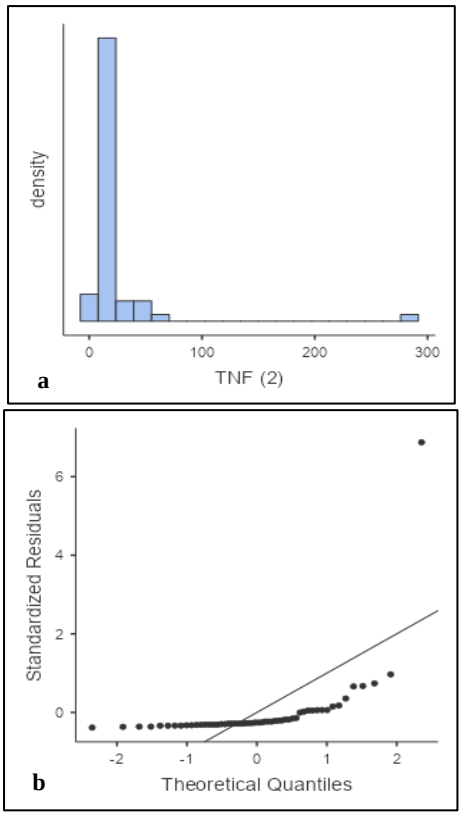

Figure 3 (a and b): TNF Alpha after 3 months.

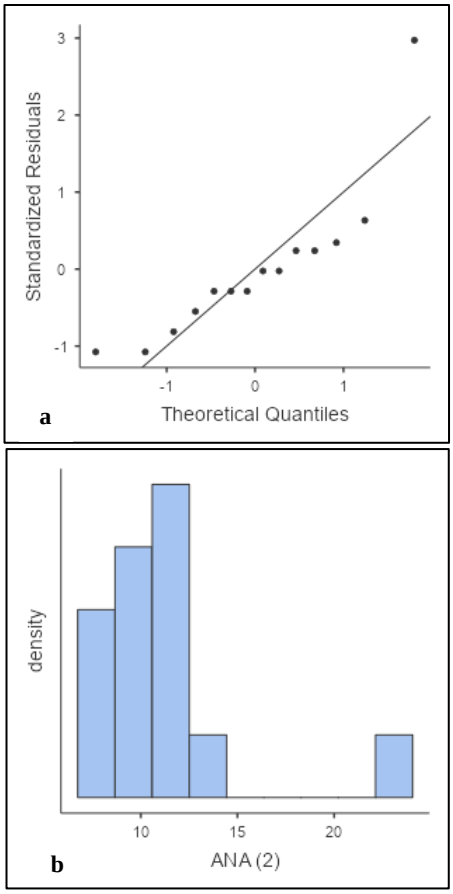

Figure 4 (a and b): ANA after 3 months.

Result of this study shows that there is a significant decrease in serum value of TNF alpha and ANA after treatment with HCQ in three months. The p value is less than 0.001 for TNF alpha and 0.001 for ANA. This is statistically significant. This shows that HCQ treatment in recurrent miscarriage patients decreases the serum value of TNF and ANA to a significant level and helps in the continuation of pregnancy. If starts HCQS as early as possible then it decreases the chances of first-trimester pregnancy loss as shown in the result of this study.

DISCUSSION

This study was aimed to find out the effect of HCQ in RPL patients who have raised TNF alpha and anti-nuclear antibody. The objective was to find out the effect of HCQ on the serum level of TNF alpha and antinuclear antibody in recurrent miscarriage patients in their current pregnancy and pregnancy outcome.

There is no definitive treatment for recurrent pregnancy loss patients, irrespective of their thrombophilia status. To date, tender loving care is the only treatment available. Chances of miscarriage increases as the age of the female increases. Another option available are intravenous immunoglobulin therapy and IVF is costly procedure and all patients cannot afford this. There is an urgent need to find other treatment options which are safe and affordable

to most of the patients. In recurrent miscarriage patients irrespective of their thrombophilia status, with increased antiphospholipid antibody but without any episode of thrombosis or any bad obstetric events, treatment with ecosprin and low molecular weight heparin is not satisfactory. Treatment benefits with the use of HCQ is not demonstrated in clinical trials.[29]

Results of this study and study done by Pasquier et al in 2019 show that the safety profile of HCQ in pregnancy and the low cost of treatment with HCQ strongly recommend the use of HCQ in recurrent pregnancy loss patients irrespective of their thrombophilia status.[30]

In a study done in 2023 by Mamta HCQ was started in recurrent pregnancy loss patients in the preconception period and continued till 20 weeks and they concluded in result that those patients taken HCQS in pre conception period conceive early and the pregnancy continued uneventful in most of the patients.[31] Result of current studies showed that HCQ causes a decrease in serum value of TNF Alpha and ANA, in this way this help in continuation of pregnancy .result of study done by Chakrabarti et al Huybrechts et al also support this finding.[32,33] In this study, 4 patients had pregnancy loss in different- different gestation ages. Rest patients had uneventful pregnancies and healthy babies. One baby had a cleft lip, which was repaired. The FALCO registry analysed 100 pregnancies in women with RPL exposed to HCQ. Women with at least three early miscarriages were included. Concomitant medications like prednisone, aspirin, and LMWH were allowed. The primary outcome was pregnancy continuation beyond 12 weeks.[34]

The authors propose that hydroxychloroquine (HCQ) may be a promising treatment for recurrent miscarriage (RM) and preeclampsia (PE) due to its immunomodulatory and vascular protective properties. Both conditions are associated with vascular dysfunction and immune system abnormalities. HCQ has shown positive effects in systemic lupus erythematosus (SLE), suggesting its potential benefits for RM and PE.[35]

The limitations were that the sample size was small and it was a single centre study.

CONCLUSION

Results of this study showed that HCQ can be used in recurrent pregnancy loss patients in their current pregnancy, who have raised values of TNF alpha and antinuclear antibody in ongoing pregnancy. Treatment should start as early as pregnancy is diagnosed.

Recommendations

Larger studies with more study participants should be conducted to support the results of the study.

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