

# Role of Serum and Placental Tissue IL6 in Unexplained Recurrent Pregnancy Loss

Dr. Jyotsana Pandey<sup>1</sup>, Prof Madhu Jain<sup>2</sup>, Dr. Shuchi Jain<sup>3</sup>

<sup>1</sup>Specialist Obstetrics and Gynaecology

<sup>2</sup>Ex HoD, Obstetrics and Gynaecology Department, BHU

<sup>3</sup>Associate Professor, Department of Obstetrics and Gynaecology BHU

**Abstract:** *The incidence of recurrent spontaneous abortion (RSA) showed an increasing trend, its pathogenesis is still unclear. At present, it is considered that the pathogenic mechanisms were related to antiphospholipid antibodies (APA) syndrome, which was mediated by the APA induced autoantibody and cause implantation failure. IL6 play an important role in cell-mediated immunity.*

**Keywords:** recurrent spontaneous abortion, pathogenesis, antiphospholipid antibodies, implantation failure, IL6, cell-mediated immunity

## 1. Introduction

“A miscarriage can be defined as the spontaneous loss of a pregnancy before the fetus has reached viability at 24 weeks (1). Only 1% women are affected by three or more consecutive miscarriages while this figure rises to 5% if RPL is defined to be 2 or more consecutive miscarriages. Proposed etiologies for RPL include uterine anatomic abnormalities, antiphospholipid antibody syndrome, endometrial infections, endocrine abnormalities, alloimmunity, genetic causes and exposure to environmental agents. However in 50% cases the etiology remains unknown and these are labeled as unexplained RPL cases (5)

However IL-6 is a pro inflammatory cytokine produced by mononuclear phagocytes, endothelial cells, fibroblasts and T cells and has many functions and effects [2]. IL-6 is involved in immune activation, vascular wall function and modulation of TNF- production. IL-6 are important in pregnancy outcome [2, 3]. IL-6 supports placental and fetal development. Hence we conducted this study to study the role of IL6 in serum and placental tissue in cases with unexplained recurrent pregnancy loss and Predicting cutoff value for serum and tissue IL6 in cases of unexplained recurrent pregnancy loss.

## 2. Materials and Methods

The present study was carried out in Department of Obstetrics and Gynaecology, Institute of Medical Sciences, Banaras Hindu University, and in Department of Molecular and Human genetics, Banaras Hindu University. The study group consists of 50 patients with early pregnancy loss, attending outdoor and hospitalized in obstetrics and gynaecology ward SSH BHU. Before inclusion in this study, objective evidence of past and present pregnancy was asked which included original lab reports of positive hCG test, ultrasound report of pregnancy. Placental tissue were collected by dilatation and curettage. Expression analysis of IL6 was done in patients 100 patients.

### a) Inclusion criteria:

Cases:

Patient attending antenatal clinic of Obstetrics and Gynaecology Department, Institute of Medical Sciences and S.S. Hospital, BHU, age 18-40 years with spontaneous abortion under 20 weeks of gestational age. The diagnostic procedures which will be used are mentioned below.

- Detailed history & clinical examination
- Urinary pregnancy test. Ultrasonography

### Control:

50 Fertile females of comparable age group (18-40 years) from same geographical locality with Amenorrhoea and positive urinary pregnancy test & sonographic evidence of intrauterine pregnancy and want medical termination of pregnancy under family planning programme.

### b) Exclusion criteria:

All those suffering from diabetes, Hypertension, Thyroid disorder, TORCH infection, Autoimmune disorder & Chromosomal abnormalities

### Collection of Samples

#### Collection of blood samples:

Blood samples (5ml) were collected in heparinized syringe from cases & controls. The TNF alpha content of all blood specimens was measured by a monoclonal radioimmunoassay.

**Collection of tissue samples:** Tissue sample from placenta was collected in RPMI Media (Roswell Park Memorial Institute. **Semi- Quantitative RT-PCR** - RNA was isolated from maternal decidua DNAase treatment was used followed by cDNA preparation done through applied biosystem kit .Expression profiling of TNF Alpha using specific forward & Reverse primers ,Relative expression study with control was done

### Details of the primers used in semi quantitative-RT PCR

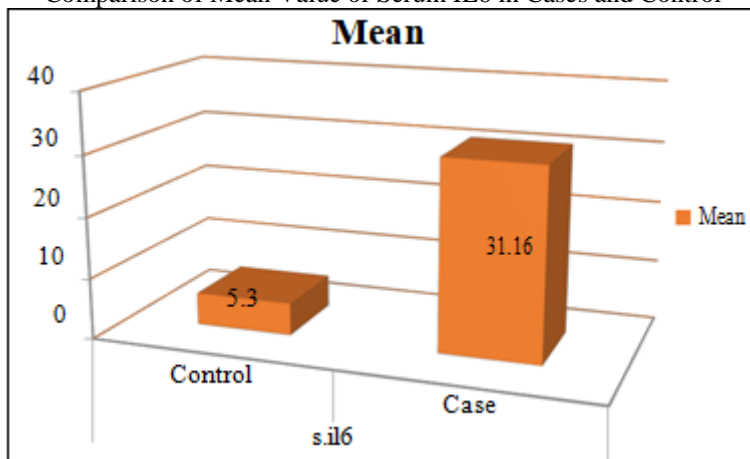
Sr No	Gene	Sequence(5' to 3')
1	Il6	Fp CAGAAGAACTCAGATGAC Rp GCTGGGCTCTGGAGGGG

3. Results

Showing mean and standard deviation, t value, p value of case and control.

Variable	Case( m+sd)	Control(m+sd)	t value	P value
s.il6	31.16±7.17	5.29±6.24	19.22	0.001
t.il6	3.90±1.69	0.89±0.47	12.03	0.001
Age	25.88±4.09	25.50±4.49	0.442	0.660

Comparison of Mean Value of Serum IL6 in Cases and Control



Comparison of Mean Value of Tissue TNF Alpha in Cases and Controls

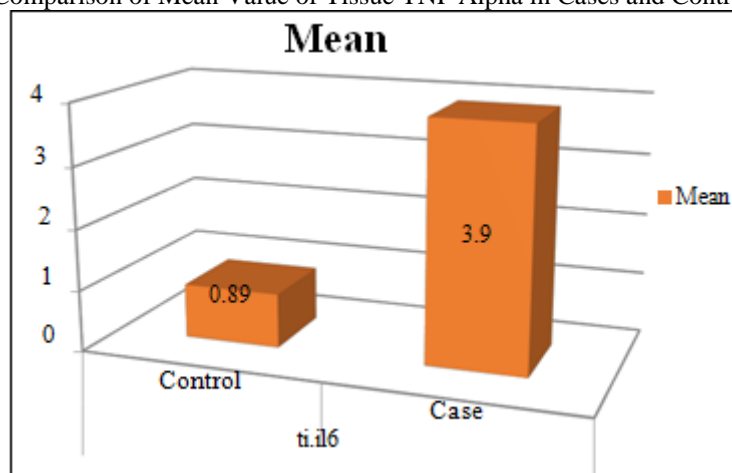
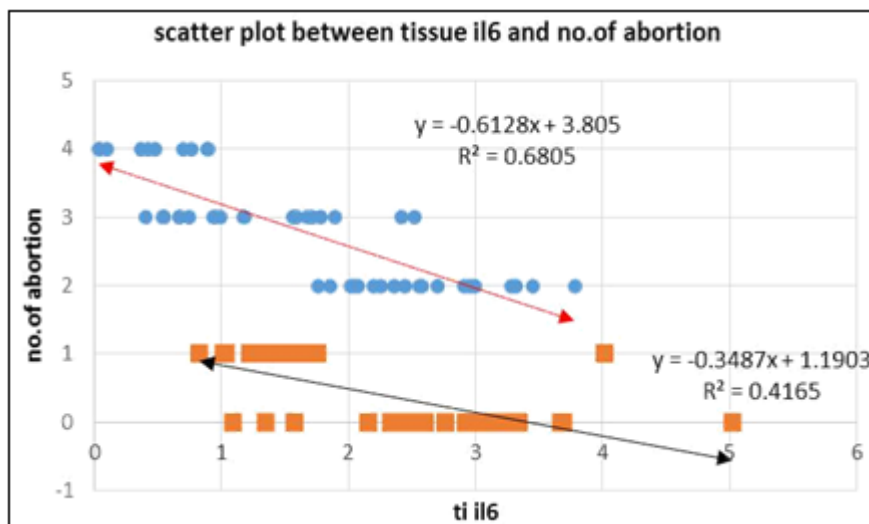
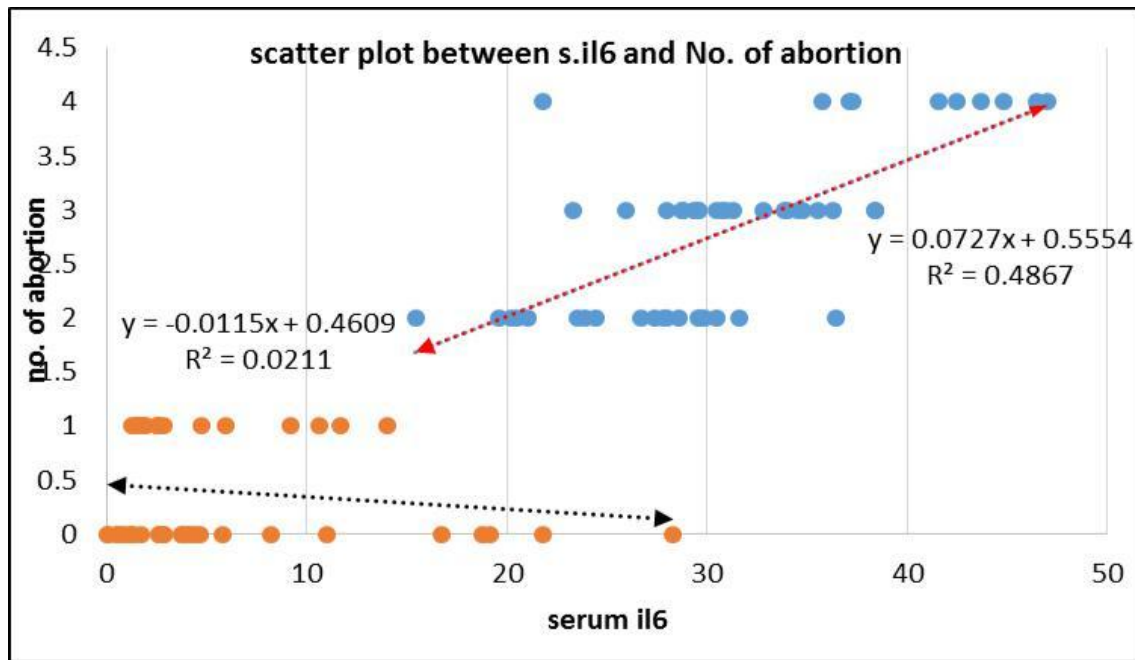


Table 1: Correlation table of RPL cases:

Variable	s.tnf alpha	s.il6	s.cox2	T.tnfalpha	t.il6	t.cox2	No. of abortion
s.il6	0.534	1	-0.507	0.646	0.613	-0.508	0.698
ti6	0.806	0.613	-0.767	0.821	1	-0.718	0.926
No. of abortion	0.848	0.698	-0.813	0.834	0.926	-0.825	1

Table showing correlation of different variables with each other in rpl patients



**Table 2:** Prediction of serum IL6 as a marker of RPL with 16.11pg/ml as a cut off

Serum IL6	Percentage
Sensitivity	98%
Specificity	90%
PPV	90%
NPV	97.8%
Accuracy	94%
Chi Square	77.93
P value	0.001

**Table 3:** Prediction of tissue IL6 as a marker of RPL with 2.02pg/ml as a cut off

Tissue IL6	Percentage
Sensitivity	98%
Specificity	98%
PPV	98%
NPV	98%
Accuracy	98%
P value	0.001

#### 4. Discussion

Spontaneous abortion, also known as miscarriage, is a frustrating and heart-wrenching experience for both the patient and the physician. Spontaneous abortion is unfortunately the most common complication of human gestation. Miscarriage is a heterogeneous condition with multiple etiological factors. So treatment needs to be individualised. Ideally all pregnancy loss should be evaluated but the high cost of medical care necessitates the implementation of certain selection criteria. RSA refers to more than two times of spontaneous abortion before 20 weeks of gestation, the incidence rate is about 0.5% - 1%[1], and with the increase of the abortion the incidence of RSA will be increased. Pregnancy failure may relate to the immune rejection of the maternal to the embryo. Therefore, it is the current research focus to clarify the mechanism of local immune regulation of the maternal-fetal interface and how to improve the abortion phenomenon. In this study, we detected the relationship of serum and placental tissue IL-6 and autoimmune recurrent spontaneous abortion

### Expression of IL6 in Serum and Placental Tissue

It has been reported that in normal pregnancy development the production of inflammatory cytokines is suppressed, whereas production of anti-inflammatory cytokines is enhanced (4). Our extensive PubMed searches have elicited no studies on endometrial expression of inflammatory cytokines during the implantation window in women with IRSM except for a study by Inagaki et al. (5) There were macrophages in decidua during each period of the pregnancy. The abnormalities of the activity and function of the macrophage can cause the imbalance of maternal fetal immune tolerance, which can lead to the occurrence of autoimmune RSA. IL-6 is a monocyte/macrophage derived cytokines, which plays a role to maintaining the uterus in a quiet state [6-8], when the balance is upset it can eventually lead to miscarriage. Our experimental results showed that IL-6 concentrations were significantly higher than the expression in the control group. The IL-6 mRNA expression in embryos tissues of the experimental group was higher than that in the control group. Immuno-histochemical results showed that both the expression were higher in the experimental group. So we can speculate that IL-6 may play an important role in the embryonic development, its high expression may be associated with autoimmune recurrent spontaneous abortion. IL-6 as an inflammatory factor have double effects, which may have immunomodulatory and anti-inflammatory and other effects in maintaining the immune tolerance of maternal-fetal. Its over expression can induced a variety of diseases and cause the miscarriage Taking 16.11pg/ml as the cut off (table 1) for serum il6 value we can detect 98% of unexplained rpl cases with 90% specificity and accuracy of 94% which is significant one. We also studied the correlation of il6 with tnf alpha and cox2 in both serum and tissue. Serum il6 has significant positive correlation with tnf alpha, both serum and tissue and also with the no. of abortion. It has a significant negative correlation with its cox2 value both in serum and tissue. Serum il6 is also significantly correlated with tissue il6 in unexplained rpl cases.

Prediction of marker of RPL with 2.02 pg/ml as the cut off (table 2) for tissue il6 value we can detect 98% of unexplained rpl cases with 98% specificity and accuracy of 98% which is significant one.

We also studied the correlation of il6 with tnf alpha and cox2 in both serum and tissue.(table 1)

Tissue il6 has significant positive correlation with tnf alpha, both serum and tissue and also with the no. of abortion. It has a significant negative correlation with its cox2 value both in serum and tissue. Tissue il6 is also significantly correlated with serum il6 in unexplained rpl cases.

### 5. Conclusion

Serum IL6 and tissue IL6 has significant positive correlation with TNF alpha, both in serum and tissue and also with the no. of abortion. It has a significant negative correlation with its cox2 value both in serum and tissue. Serum il6 is also significantly correlated with tissue il6 in unexplained rpl cases

Tissue il6 contributes maximum that is 85% of all the unexplained RPL cases, followed by tissue tnf alpha. As our study showed positive correlation between tissue and serum IL6 levels we can conclude that over expression of IL6 gene may be involved in pathogenesis of recurrent pregnancy loss. Measuring there level in patient with unexplained pregnancy loss and treating them can lead to significant decrease in incidence of recurrent spontaneous abortion.

### References

- [1] American Society for Reproductive Medicine. Evaluation and treatment of recurrent pregnancy loss: a committee opinion. *Fertil Steril* 2012;98:1103–11.
- [2] Rand JH, Wu XX, Quinn AS, AS, Taatjes DJ. The annexin A5-mediated pathogenic mechanism in the anti-phospholipid syndrome: role in pregnancy losses and thrombosis. *Lupus* 2010;19(4): 460-469.
- [3] Rai R, Regan L. Recurrent miscarriage. *Lancet* 2006; 368(9535): 601-611
- [4] Mor G, Romero R, Abrahams VM. Macrophages and pregnancy. In: Mor G, editor. *Immunology of pregnancy*. Georgetown, TX: Landes Bioscience/Springer; 2006:63–72.
- [5] Inagaki N, Stern C, McBain J, Lopata A, Kornman L, Wilkinson D. Analysis of intra-uterine cytokine concentration and matrix-metalloproteinase activity in women with recurrent failed embryo transfer. *Hum Reprod* 2003;18:
- [6] Hong Y, Lin QD. Determination of secretory phase and early endometrial macrophages and natural killer cells. *Chin Obstet Gynecol* 2000; 35(1): 36-38.
- [7] Stella CL, Bennett MR, Devarajan P, Greis K, Wyder M, Macha S, et al. Preterm labor biomarker discovery in serum using 3 proteomic profiling methodologies. *Am J Obstet Gyneol* 2009; 201(4): 387.e1-13.
- [8] Xiao SJ, Zhao AM, Bao SM. Relationship between expression of COX-2, its signal transduction pathways and autoimmune-type recurrent miscarriage. *J Shanghai Jiaotong Univ (Med Sci)* 2011;31(7): 895-899.