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RESEARCH ARTICLE

Interleukin 15 as serum biomarker for ectopic pregnancy and missed abortion

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ABSTRACT

Background: When patients with biochemically diagnosed pregnancy, presents with pain and or vaginal bleeding, the differential diagnosis between intrauterine pregnancy, missed abortion and ectopic pregnancy is challenging. There is present lack of clinically useful tests for the accurate diagnosis of ectopic pregnancy, unless an early intrauterine pregnancy (IUP) is visible by ultrasound. **Objectives:** To assess whether serum measurement of IL-15 at 6–8 weeks could contribute to the differential diagnosis between failed pregnancies (i.e ectopic pregnancy or missed abortions) and healthy intrauterine pregnancies. **Materials and methods:** We performed a prospective observational case control study of 75 patients . Among these 75 patients, 50 patients presented with early pregnancy with abdominal pain or vaginal bleeding between 6- 8 weeks of gestation and were either diagnosed as ectopic pregnancy (EP) (25) and missed abortion (MA) (25). 25 women with healthy intrauterine pregnancy (IUP) between 6-8 weeks of gestation served as the control group. Serum samples were collected at the initial visit before treatment. All diagnosis were histologically confirmed. Serum beta HCG and IL-15 were measured in all 75 patients. **Results:** The mean serum IL-15 levels were significantly higher in failed (ectopic & missed) as compared to normal pregnancies (58.51± 8.95 vs 47.90 ±9.66 pg/ml respectively)(p=0.001). However there was no significant difference in IL-15 levels between missed (58.47 ±10.94 pg/ml) and ectopic (58.91± 7.94 pg/ml) pregnancies (p=0.87). The AUC for failed vs normal pregnancy was 0.810 (p=0.000). At the IL15 reading of 49.47pg/ml, the sensitivity was 86% and the test expects 34.6% false positivity rate in distinguishing failed pregnancy (EP, MP) from normal pregnancy. However the AUC was 0.53 when we compared ectopic pregnancy with missed abortion. **Conclusion:** High IL15 levels were associated with failed pregnancies, so these patients need to be closely followed. Larger randomized controlled trials are needed to further explore the role of IL15 to distinguish ectopic pregnancy and missed abortion.

Keywords: Intrauterine pregnancy, missed abortion, ectopic pregnancy, IL15.

The accurate diagnosis of ectopic pregnancy is tricky in early stages, unless an early intrauterine pregnancy (IUP) is visible by ultrasound. When these patients with biochemically diagnosed pregnancy, present with pain and/ or vaginal bleeding, the differential diagnosis between IUP, missed abortion and ectopic pregnancy is a challenge. Where there is a fear of intervention in a desired normal intrauterine pregnancy misdiagnosed as ectopic pregnancy, there is also a danger of an ectopic pregnancy which might rupture being diagnosed as intrauterine pregnancy/missed abortion.

Ectopic pregnancy is the most common cause of maternal death in first trimester [1]. In recent times, the incidence has increased significantly and it now affects 2-3% of pregnancies [2]. Because of current diagnostic tests of beta HCG and transvaginal ultrasound, the diagnostic accuracy has improved but early diagnosis is still a challenge. During early pregnancy the immunological processes that take place are modulated by pro and anti-inflammatory cytokines. The normal intrauterine pregnancy is considered as Th1-Th2 cooperation with predominance of Th2 lymphocyte response

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and specific cytokine production [3,4,5].

IL-15 is a cytokine which is expressed by human placental tissue. The serum levels correlate with the duration of pregnancy, and it is maximally expressed during implantation in the deciduas [6, 7]. IL-15 is produced by a large variety of cells and tissues, including epithelial cell lines, monocytes, macrophages and decidual and endometrial tissues [7]. IL-15 belongs to IL-2 cytokine family. IL15 is a pleotropic cytokine and it helps in production of Th1 predominant proinflammatory cytokines [8], thus promoting T-cell and NK cell proliferation [9]. It also helps in regulation of development and killing activity of NK cells [10,11]. Further the recurrent abortion cases have an upregulation of IL-15 expression in trophoblasts, thus IL-15 can be a marker for pregnancy failure. Our focus is to study the serum levels of IL-15 in ectopic, missed abortion and normal intrauterine pregnancy.

Aims & objectives: To assess whether IL-15 serum measurement at 6-8 weeks could contribute to the differential diagnosis between failed pregnancies, whether ectopic or missed abortions, and healthy intrauterine pregnancies.

Materials and methods

We performed an observational case control prospective study of 75 patients in the department of Obstetrics and gynaecology in Maternity hospital of SKIMS, Soura, Srinagar. Among these 75 patients, 50 patients presented with early pregnancy with abdominal pain or vaginal bleeding between 6- 8 weeks of gestation and were either diagnosed as ectopic pregnancy (EP) (25) and missed abortion (MA)(25). 25 women with intrauterine pregnancy (IUP) between 6-8 weeks of gestation served as the control group. This study was conducted between Jan 2018 to March 2019.

Serum samples were collected at the initial visit before treatment. If the clinician was unable to make a diagnosis on the first visit, the patient was admitted and followed up until a diagnosis of a viable intrauterine pregnancy or MA or EP was confirmed. All diagnosis were histologically confirmed. Serum beta HCG and IL-15 were measured in all 75 patients. All participating individuals gave informed consent for the study .

IL-15 ELISA measurements: Serum samples were collected at the initial visit before treatment. All samples were processed by centrifuge (1,000 g for 15 minutes), and the supernatants were stored at -80^0 C until assayed. Serum concentrations of IL-15 were determined by quantitative sandwich ELISA (Human IL-15 ELISA kit, Diaclone. Cat no. 850-006-096) according to the instructions of the manufacturer. Uman IL-15 is a quantitative sandwich enzyme immunoassay technique based on a monoclonal antibody specific for IL-15 which has been precoated onto a microplate. Testing steps have been followed in according to the protocols provided by the manufacturer.

Statistical methods: Standard statistical procedures were used to analyze the data. Data were described as mean ± standard deviation and percentages. ANOVA and Fisher's exact test were used to calculate *P* values. SPSS 20.0 (IBM SPSS Statistics for Windows, IBM Corp, Armonk, NY, USA) and Microsoft Excel software were used for data analysis. $P < 0.05$ was considered statistically significant.

Results

The mean age of entire cohort was 30.3 years. The mean age of EP was 32. The mean age of MA was 31.5 years .The mean age of IUP was 27.5 years. The mean Beta HCG levels in ectopic pregnancies was 2068.5(326-3500) mIU/ml. The mean Beta HCG levels in missed pregnancies was 4058.5(826-8000) mIU/ml. The mean serum IL 15 in ectopic pregnancies was 58.91± 7.94 pg/ml.

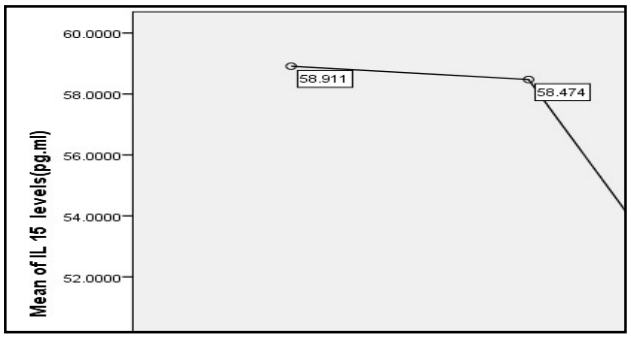

Figure 1: Showing mean IL15 levels in normal vs ectopic vs missed pregnancies

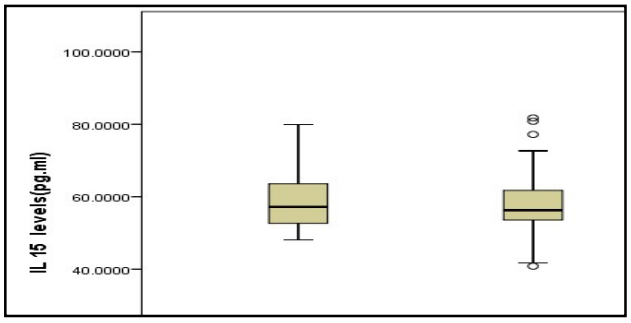

Figure 1: Box plot showing IL15 levels in various subsets

The mean serum IL-15 in missed pregnancies was 58.47 ±10.94 pg/ml while as it was 47.90 ±9.66 pg/ml in normal pregnancies (p=0.001). The mean serum IL15 levels were

significantly higher in failed (ectopic & missed) as compared to normal pregnancies (p=0.001) (figure 1, 2). However there was no significant difference in IL-15 levels between missed (58.47 ±10.94 pg/ml) and ectopic (58.91± 7.94 pg/ml) pregnancies (p=0.87) (figure 1, 2).

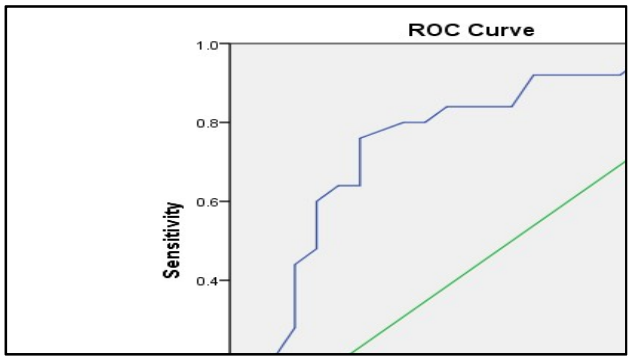

Figure 3: ROC curve normal vs missed pregnancies, area under curve 0.787

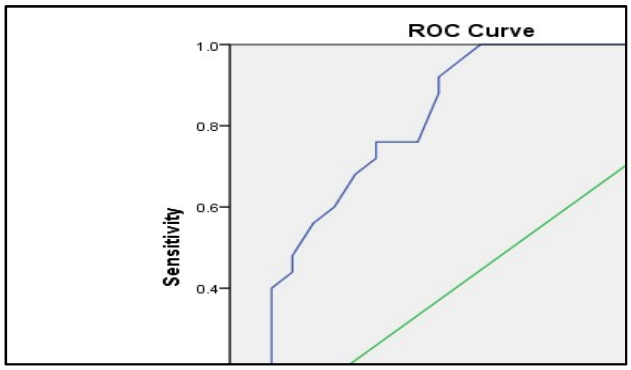

Figure 4: ROC curve normal vs ectopic pregnancies, area under curve 0.827

IL-15 values were plotted in ROC curves in order to further evaluate their diagnostic accuracy for the diagnosis of healthy IUP and for accurate discrimination of healthy IUP from failed pregnancy (EP, MA). When we compared missed vs normal pregnancy, AUC for IL-15 was 0.787 (p=0.000) (figure 3). At the IL15 reading of 51.29 pg/ml, the sensitivity was 80% and the test expects 30.8% false positivity rate in distinguishing missed abortion from normal pregnancy. The AUC for ectopic vs normal pregnancy was 0.827 (p=0.000) (figure 4). At the IL-15 reading of 49.47pg/ml, the sensitivity was 92% and the test expects 37% false positivity rate in distinguishing ectopic pregnancy from normal pregnancy. The AUC for failed vs normal pregnancy was 0.810 (p=0.000) (figure 5). At the IL-15 reading of 49.47pg/ml, the sensitivity was 86% and the test expects 34.6% false positivity rate in distinguishing failed pregnancy (EP, MP) from normal pregnancy. However the AUC was 0.53 when we compared ectopic pregnancy with missed abortion (figure 6).

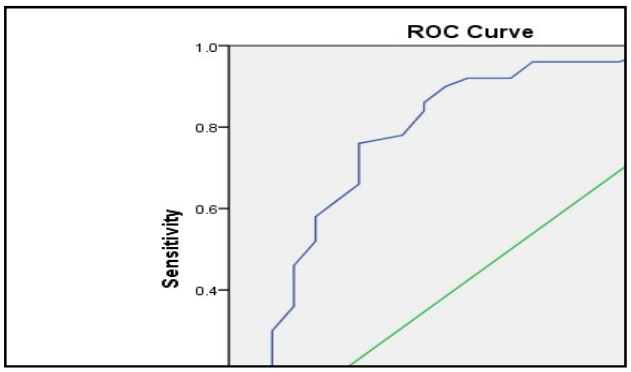

Figure 5: ROC curve normal vs failed pregnancies, area under curve 0.81

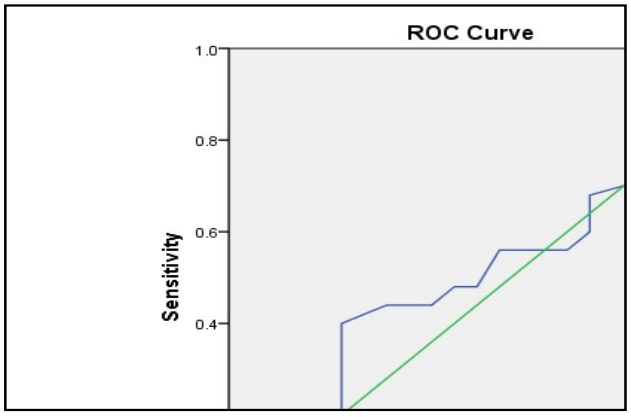

Figure 6: ROC curve ectopic pregnancy vs missed abortion, area under curve 0.53

Discussion

The biomarker reflecting the viability of a pregnancy differentiates normal viable intrauterine pregnancy from abnormal pregnancy (i.e ectopic pregnancy or missed abortion). Such biomarkers fail to differentiate an ectopic pregnancy from missed abortion. Thus the markers reflecting the location of pregnancy, rather than viability of conceptus, will obviously help to differentiate between the two types i.e ectopic pregnancy and missed abortion.

Our analysis has shown that ectopic pregnancies and missed abortions had increased IL-15 levels as compared to normal intrauterine pregnancy and the difference between the two was statistically significant. However it could not differentiate between ectopic pregnancy and missed abortion

- the values being high in both as compared to normal intrauterine pregnancy. This was comparable to Toth et al [12] which reported that IL15 expression is upregulated in placental tissue of disturbed first trimester pregnancy and the trophoblast cells were detected as main source of IL-15. Though IL-15 is abundantly expressed in non pregnant uterus and in uteroplacental unit during pregnancy [13] its unrestrained expression is associated with spontaneous and recurrent miscarriages [14,15]. Our study differed from Dapone et al [16]. When compared to Dapone et al, our study was comparable in terms of difference in IL15 between ectopic pregnancy and live intrauterine pregnancy which was statistically significant but it differed in terms of difference in IL-15 between ectopic pregnancy and missed abortion. This topic being still under research will need larger prospective study to confirm if there is a difference in IL-15 levels between ectopic pregnancy and missed abortion cases. It thus needs further confirmation if IL-15 levels reflect the viability or location of pregnancy.

In ectopic pregnancies, the trophoblasts which invade the tube or peripheral NK cells can account for the increased IL-15 levels as compared to normal intrauterine pregnancy but this certainly needs further exploration. However, the aim of our study was not to explore if IL15 is directly or indirectly related to the development of ectopic abortion and missed abortion. Thus it is uncertain if increased IL15 levels are the cause or effect of pregnancy failure.

IL15 levels greater than 49 pg/ml have 86% sensitivity in distinguishing failed from IUP pregnancies and hence women with bleeding is early pregnancy with no visible gestational sac with IL 15 levels greater than 49 pg/ml need to be closely followed while as those with lower levels could be scheduled for regular antenatal visits. The women with low IL 15 levels may not be admitted as possible pregnancy failures with obvious cost benefits.

The research on utility of IL-15 levels in ectopic pregnancy needs to be tested in a longitudinal cohort study to find its clinical utility. We hope that our study contributes to the research that is underway for identifying novel single /multiple biomarkers with the aim of accurately diagnosing ectopic pregnancies.

Conclusion

High IL15 levels were associated with failed pregnancies, so these patients need to be closely followed. Larger randomized controlled trials are needed to further explore the role of IL15 to distinguish ectopic pregnancy and missed abortion.

Conflict of interest: None. **Disclaimer:** Nil.

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