IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 14, Issue 1 Ver. IV (Jan. 2015), PP 74-78
www.iosrjournals.org

Doppler Blood Flow Studies of the Endometrium and Its Relation with Serum VEGF in Women with Unexplained Infertility

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Abstract:
Aim: *Angiogenesis is an essential prerequisite for endometrial development and differentiation. Absence of optimum endometrial perfusion can be one of the important reasons behind the patients with unexplained infertility. The present study was undertaken to identify the endometrial vascularity by Doppler and find its relation with serum VEGF in infertile women.*
Materials And Methods: *60 Patients who qualified the exclusion and inclusion criteria were enrolled in the study. Patient's blood was obtained by venepuncture on the day of Doppler examination to be evaluated for serum VEGF.*
Results: *Doppler vascular penetration zones were identified as zone 1, zone 2 and zone 3 i.e. poor, intermediate and good vascularity respectively. A rise in the mean serum VEGF level was observed with the increase in the endometrial vascular penetration zones on Doppler i.e. from 37.67±10.53 pg/ml in Zone 1 to 83.69 ±19.86 pg/ml in Zone 2 to 215.07±25.60pg/ml in Zone3($p<0.05$). The Doppler vascular penetration zones were taken as gold standard and cut-off value of serum VEGF was determined that could predict good endometrial receptivity.*
Conclusion: *Serum VEGF levels were found to rise with increasing Doppler vascular penetration zones which implies that serum VEGF concentrations can be used as a marker of endometrial receptivity.*
Keywords: *Receptivity, Doppler, VEGF, Endometrium, Infertility*

I. Introduction

Infertility in itself represents a wide spectrum of disorders explaining the inability to conceive. Series of investigations are required to depict the potential cause of infertility, yet there exist a small percentage (10-25%) of couples in which no obvious cause is delineated. These groups of patients are classified as under 'unexplained infertility' [1, 2]. The probable cause and subsequent management remains an enigma in this group. The endometrium provides a receptive milieu for the fertilized ovum whose role in determining fertility can never be underestimated. Through the menstrual cycle there is increase in its thickness, change in pattern to secretory and enhancement in its vascularity [2]. These changes make it receptive for the blastocyst. The molecular regulation of the various mechanism involved in implantation and gestation are exquisitely controlled & remarkably complex [3]. Angiogenesis supports endometrial proliferation and transforms thin dense endometrium into thick, highly permeable secretory endometrium. It is possible that cohort of women presenting with unexplained infertility have decreased uterine and endometrial perfusion[2-5], which might be controlled directly or indirectly by reproductive steroids and angiogenic regulators like Vascular Endothelial Growth Factor (VEGF).

The present study was undertaken with an aim to identify the endometrial vascularity by Doppler and find its relation with serum VEGF in infertile women.

II. Materials And Methods

The present study was conducted in the Department of Anatomy in collaboration with Department of Obstetrics & Gynaecology and Department of Pathology of Lady Hardinge Medical College and Smt. Sucheta Kriplani hospital, New Delhi. 150 patients attending gynaecology OPD with history of primary or secondary infertility were considered for the study. These patients were subjected to routine investigations as per the hospital's protocol. Husband's semen analysis was done as per standard WHO guidelines to rule out male factors of infertility. This was followed by routine haematological examinations i.e. complete blood count with ESR, blood sugar, VDRL, liver and kidney function test was done to rule out metabolic factors responsible for infertility. Routine Transabdominal Ultrasonography (TAS) was done for all female patients to rule out known

causes of infertility like uterine malformations, Fibroids, Asherman's syndrome, tubercular endometritis and tubal blockage. Tubal patency was determined by HSG (Hystero-salpingography).

After these preliminary investigations 60 patients with unknown causes of infertility were included in the study group. This included patients in the age group of 20-35 yrs with varied duration of infertility [Fig-1] comprising the cases of both primary as well as secondary infertility [Fig-2]. These patients were then followed with Doppler ultrasonographic examination with a 5 MHz endovaginal probe using Nemio XG SSA-580A (Toshiba), which was performed during secretory phase of the endometrial cycle [day 24/ 25].

Patient's blood was obtained by venepuncture on the day of Doppler examination to be evaluated for serum VEGF. The assay was done using DIACLONE VEGF-A BIOLISA Kit (quantitative) which measures free or active VEGF-A in serum. The antibodies used in this ELISA test were specific for human VEGF with no detectable cross-reactivity with placental growth factor.

III. Observations And Results

Doppler vascular examination is the most objective method of identifying endometrial vascularity. Based on the degree of vascular penetration into the endometrial thickness, the endometrium was classified into three different zones [6].

Zone 1(poor vascularity): Doppler colour signals i.e. the vascular penetration present only in the myometrium.

Zone 2(intermediate vascularity): vascular penetration present in the outer half of the endometrial thickness, not reaching till its innermost lining.

Zone 3(good vascularity): vascular penetration present in whole of the endometrium i.e. till its innermost endometrial lining[Fig 3].

The total numbers of patients were now distributed according to the Doppler zones of vascularity [Fig-4]. The mean serum VEGF of patients falling in different zones of Doppler vascularity was calculated. A rise was observed in the mean serum VEGF level with the increase in the endometrial vascular penetration zones on Doppler i.e. from 37.67±10.53 pg/ml in Zone 1 to 83.69 ±19.86 pg/ml in Zone 2 to 215.07±25.60pg/ml in Zone3. The difference of mean serum VEGF level in different vascular penetration zones i.e. Zone 1, Zone 2 and Zone 3 was found to be highly significant($p=0.000$) [Fig-5].

The Doppler vascular penetration zones were taken as gold standard and cut-off value of serum VEGF was determined that could predict good endometrial receptivity. Using the Receiver operator curve (ROC), it was found that the serum concentration of 87pg/ml was able to predict good endometrial receptivity with a sensitivity of 89.47% and a specificity of 87.8%. The positive predictive value of the test was found to be 77.27% and negative predictive value was 94.73%. The area under the curve was 0.943 with a standard error of 0.031, $p=0.000$. [Fig-6]

IV. Discussion

Angiogenesis is an essential prerequisite for human endometrial development and differentiation, further for the implantation of blastocyst and continuation of pregnancy. The absence of sub-endometrial perfusion is associated with non-conception cycles [7,8]. The crucial role of angiogenesis on human endometrium was hypothesised by Klauber et al in their study on effect of inhibitors of angiogenesis on endometrial growth. An angiogenesis inhibitor AGM-1470 was injected in both pregnant and non-pregnant cycling mice; in pregnant mice interference with decidualization, placental and yolk sac formation and embryonic vascular development was seen that resulted in complete failure of embryonic growth. While in non –pregnant mice it resulted in inhibition of endometrial maturation and corpus luteum formation. It implies that angiogenesis directly influences endometrial maturation and endometrial receptivity for embryo implantation [9, 10] and its importance in determining fertility cannot be overlooked. Among the several known regulators of angiogenesis, vascular endothelial growth factor is one of the major regulators of endothelial cell proliferation, angiogenesis, vasculogenesis and capillary permeability [11-13]. Its levels increase around the peri-implantation period and act as key regulator of angiogenesis and vascular function in the human endometrium. Alterations in the levels of this factor can cause aberrant endometrial angiogenesis that may result in failure of implantation and recurrent miscarriages [11, 14, 15].

Doppler is an excellent non invasive method to document the blood flow in the endometrium. Doppler studies of the endometrium and serum levels of VEGF both indicate the level of perfusion of the endometrium. In the present study, zone 3 vascularity was seen in 21.7 % patients, zone 2 in 43.3% & zone 1 in 35% patients. Of these 4 patients out of 60 who subsequently conceived had good vascularity i.e. vascularity reaching till the innermost endometrial lining (mean VEGF levels 225±20.50pg/ml of the patients who conceived as compared to 203.33±24.96 pg/ml in the patients who didn't, in the good vascularity group). No conception was noted in the patients with poor or intermediate vascularity group as identified in Doppler vascular zones 1 and 2.

Similar to the observations of the present study, Chien et al in their study on assessment of uterine receptivity by the endometrial-subendometrial blood flow distribution pattern in women undergoing in vitro fertilization-embryo transfer noticed a significantly higher pregnancy and implantation rates in patients with increased vascularity i.e. when vascular penetration was seen till the innermost endometrial lining or inner-half of the endometrium as compared to the patients in which there was no detectable blood flow on Doppler imaging [16].

Unkila-Kallio et al did not observed any change in serum VEGF levels during normal menstrual cycle, though a significant mid luteal phase elevation was seen in patients undergoing In Vitro Fertilization programme [17]. Agarwal et al measured serum VEGF levels in 14 healthy volunteers in early follicular phase, pre-ovulatory and midluteal phase. They reported that the levels of serum VEGF were highest in the midluteal phase [18].Therefore serum VEGF levels and Doppler blood flow studies were measured in midluteal phase of the cycle i.e. at time when maximum blood flow to the endometrium is expected.

In the present study, when the serum VEGF levels were correlated with zones of vascularity as estimated by Doppler blood flow studies it was found that there was a marked correlation between serum VEGF levels and blood flow in the endometrium by Doppler. It was observed that the mean serum VEGF levels were significantly higher in patients with Zone 3 vascularity i.e. vascularity present in the whole of endometrium. On the other hand the serum VEGF levels were lowest in patients with Zone 1 vascularity and levels were intermediate in patients with Zone 2 vascularity. These results are in agreement with the study of Agarwal et al on the association of serum VEGF in normal menstrual cycle with changes in the ovarian and uterine blood flow who observed a concurrent rise in serum VEGF concentration with the peak systolic velocity (PSV) in ovarian and uterine arteries [18] implying that serum VEGF concentrations can be used as a marker of endometrial receptivity.

V. Disclosure

The study was supported by Lady Hardinge Medical College, New Delhi. Authors haven't received any financial aid from any of the companies and have no financial relation with any of the firms.

VI. Figures and Tables

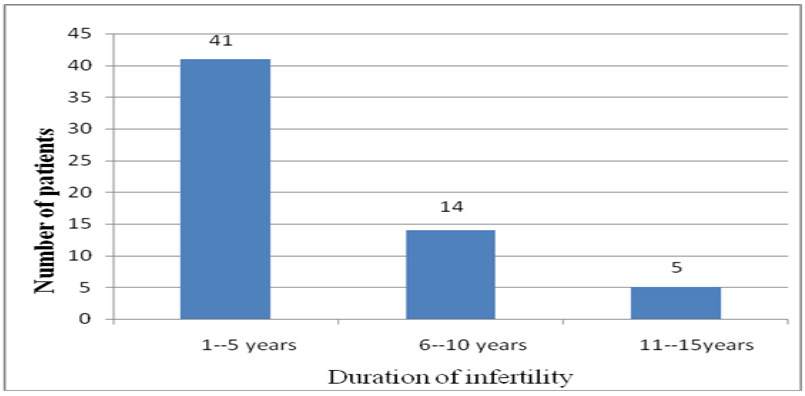

Fig 1- Distribution of patients with infertility duration

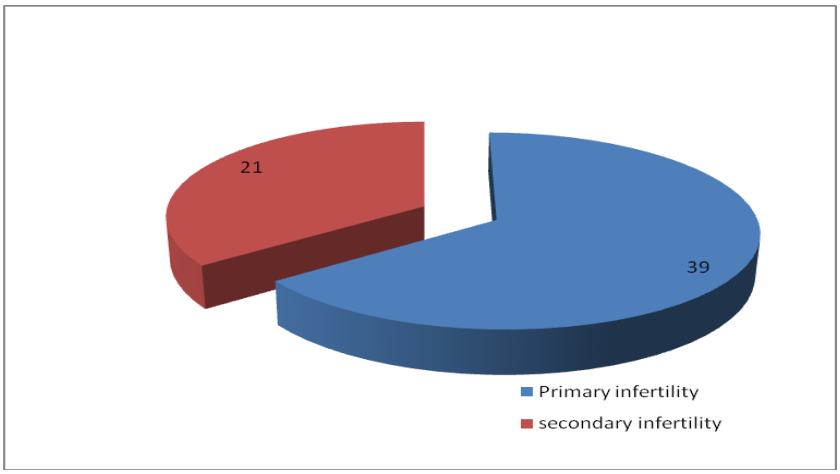

Fig2- Distribution of patients in different types of infertility

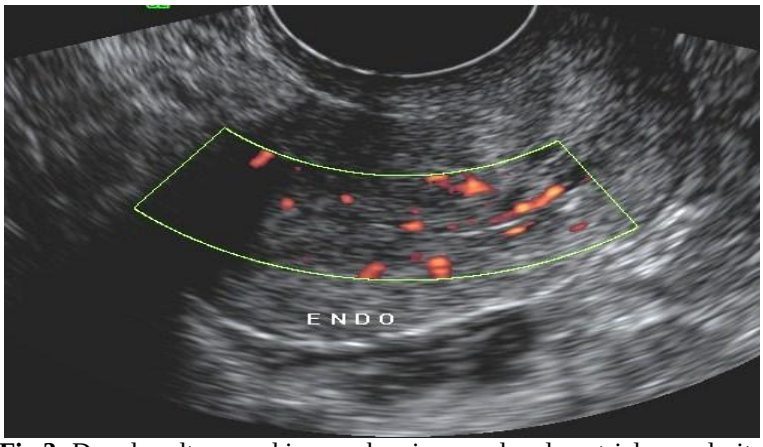

Fig 3- Doppler ultrasound image showing good endometrial vascularity

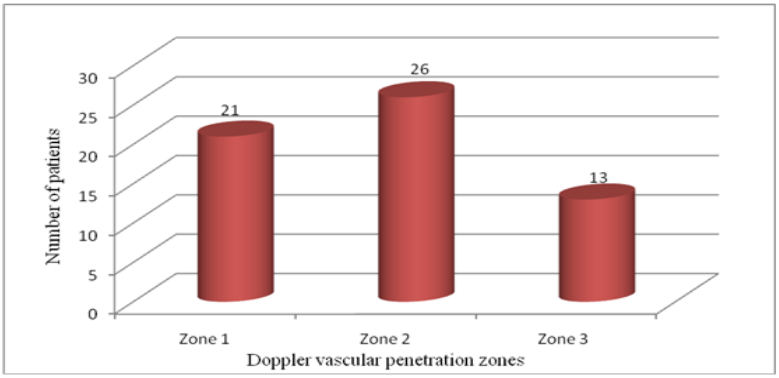

Fig 4- Distribution of patients in Doppler vascular penetration zones

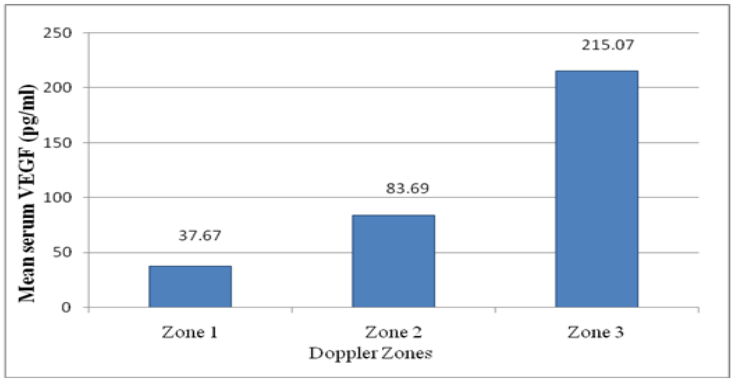

Fig 5 - Serum VEGF levels in Doppler vascular penetration zones

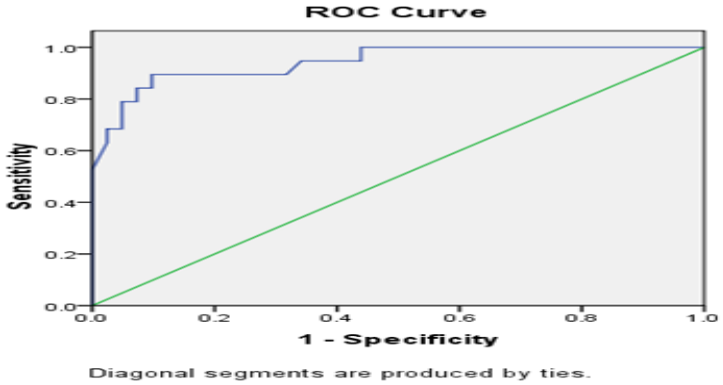

Fig 6- Receiver operator curve to determine cut off of serum VEGF based on Doppler study

VII. Conclusion

Vascular endothelial growth factor (VEGF) is a major regulator of endothelial cell proliferation, angiogenesis, vasculogenesis and capillary permeability. A concurrent rise in the serum VEGF level was observed with increase in the Doppler vascular penetration zones. A receptive endometrium is a reflection of good endometrial vascularity, which signifies serum VEGF as a marker of endometrial receptivity.

Acknowledgements

The authors are thankful to the technical staff of pathology and anatomy department of Lady Hardinge Medical College for their constant cooperation. A special thanks to Dr. Manisha Hansda for her constant support and encouragement.

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