AN IMMUNOHISTOCHEMICAL EVALUATION OF SFLT-1 IN SYNCYTIAL KNOTS OF HUMAN PREECLAMPTIC PLACENTA:

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ABSTRACT:

Objective: The study was designed to determine the expression of sflt-1 in syncytial knots of preeclamptic placentas compared with normal placentas. Materials and Methods: Immunohistochemical staining for sflt-1 was performed on 50 normal and 50 preeclamptic placental tissues and the level of expression was quantified by semiquantitative method. Results: The sflt-1 expression was significantly higher in syncytial knots of preeclamptic placenta compared to that of control. Conclusion: It was concluded that the syncytial knots are the placental source of sflt-1 secretion in preeclampsia.

Key words: *Placenta, Preeclampsia, Syncytial knots, Soluble fms like tyrosine kinase(sflt-1).*

INTRODUCTION:

Preeclampsia is a systemic disorder which is clinically manifested with new onset of maternal hypertension, proteinuria and edema at 20 weeks of gestation. Preeclampsia affects 5-7% population worldwide causing morbidity and mortality to both mother and the fetus. The only successful treatment is delivery of placenta (Sibia B et al 2005).

Recent research has shown that soluble fms-like tyrosine kinase-1(sflt-1) is one of the key "toxic factors" released by the placenta into the maternal circulation and that it contributes to the hypertension, proteinuria and edema associated with this disorder (Clark DE et al 1998).

sflt1 is a soluble form of the vascular endothelial growth factor receptor that lacks the cytoplasmic tail and transmembrane domain but retains the extracellular ligand – binding domain. sflt-1 or sVEGFR1 prevents circulating VEGF and PLGF interactions with their proangiogenic receptors and functions as an antiangiogenic factor. The level of sflt-1 in the plasma of women with preeclampsia is elevated in comparison with that of women with uncomplicated pregnancies (Koga K et al 2003, Levine RJ et al 2004).

The placenta has two types of trophoblast cells covering the chorionic villi namely the inner cytotrophoblast and outer syncytiotrophoblast. On the surface of the syncytiotrophoblast layer, the aged and late apoptotic syncytiotrophoblast nuclei are packed into apical protrusions called syncytial knots (Benirschke K et al ;2006). These syncytial knots are released into the maternal circulation and are transported to the lungs through maternal venous system. In the lungs, the syncytial knots are engulfed by lung macrophages. This shows that the syncytial knots are the only placental material that enters into the systemic circulation (Redman CW and Sergeant IL;2008).

In the present study we found increased number and sflt-1 staining of syncytial knots in preeclamptic placenta compared to normal. We hypothesized that excess syncytial knots shed into the maternal circulation are the major source of increased circulating sflt-1 in preeclampsia.

MATERIALS AND METHODS:

A total of 100 placental tissues from uncomplicated and preeclampsia pregnancies were included in the study. These were collected from the SRM University, Hospital, Department of Gynecology and Obstetrics. Informed consent forms and protocols were approved by the institutional ethical committee. Placental tissues were divided in two study groups. The control/normotensive group consisted of placental tissues collected from 50 women with uncomplicated pregnancies who had normal blood pressure and no proteinuria. The preeclampsia group contained tissues collected from 50 women in whom preeclampsia was defined as a blood pressure of >140/90mm Hg, and with proteinuria >300mg/l in a 24hr urine collection. Samples (1.5x1.5x1cm in diameter) taken from the maternal surface of each placenta; infarct areas were excluded from the study.

IMMUNOHISTOCHEMISTRY:

Paraffin sections were mounted on glass slides, dewaxed in xylene, and rehydrated in descending ethanol gradient. Antigen retrieval was performed by heating in sodium citrate solution (10 mmol). Endogenous peroxidase was quenched with 3% (vol/vol) hydrogen peroxide in PBS for 30 min. After blocking (5% normal goat serum for 1 h), the slides were incubated overnight with primary antibody (anti-human soluble VEGFR-1, 1:150 dilution). Slides were washed in 1×PBS and exposed to peroxidase-conjugated secondary antibody (1:300, goat anti-rabbit, Vector Laboratories) for 45 min at room temperature. Finally, avidin biotin complex (Vector Laboratories) was applied for 1 h, and staining was

detected with the diamino-benzidine chromogen after 5 min. Slides were counterstained with hematoxylin.

Evaluation of immunohistochemical staining:

The intensity of the staining reaction of sflt1 in Syncytial knots was evaluated by two investigators blind to the purpose of the study. Immunoreactivity for antibodies was scored using a semi-quantitative scale for intensity of staining: 0 negative, no staining; 1+ weak positive; 2+moderately positive; 3+ strongly positive. The numbers of syncytial knots were counted randomly in 5 fields per section by two investigators who are blinded to the study.

Statistical analysis:

The intensity of sflt1, for each slide, a value designated 'H score' was obtained by application of the following algorithm score" =Σ(I×PC), where I and PC represent intensity and percentage of cells that stain at each intensity, respectively and corresponding H- score were calculated separately.

Statistical analysis was carried out using SPSS for windows (version 13.0, Chicago IL, USA). Data were expressed as mean ± standard deviations. The differences of groups were analyzed by Mann-Whitney U test. P value < 0.001 was considered statistically significant.

RESULTS:

The number of syncytial knots increased in preeclamptic placenta compared with control (11.77±1.44 and 6.4±1.30 respectively). The expression of sFLT-1 in syncytial knots were significantly higher in preeclamptic placenta compared with control (250±18.38 and153±37.78 respectively). Fig1(A&B).

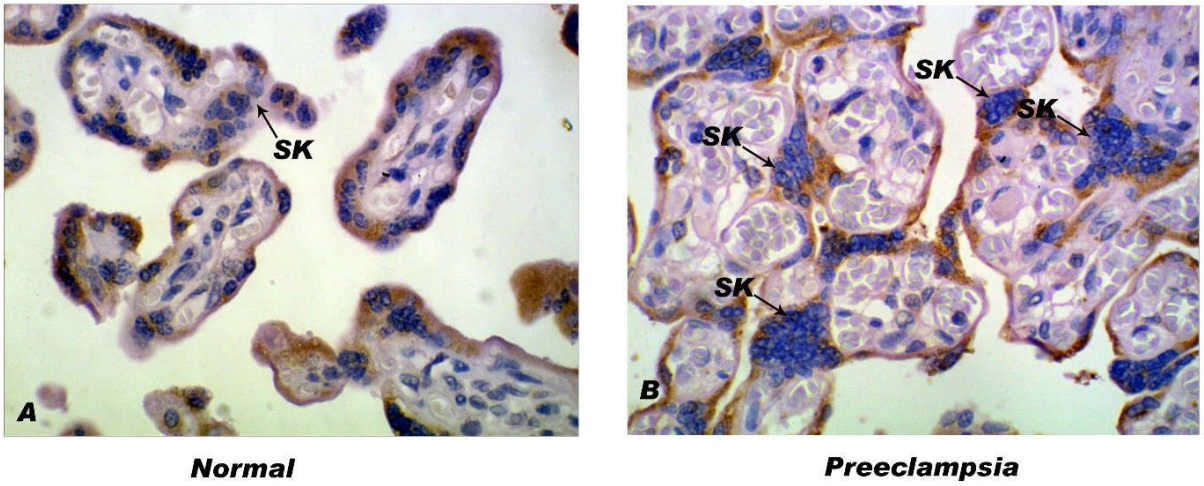

Figure1. Increased number and sflt-1 staining in syncytial knots of preeclampsia placenta (A & B)

DISCUSSION:

The syncytial knots are accumulation of nuclei attached to the tips of placental villi. Normally these syncytial knots break off and drop into the intervillous blood lakes and enter the maternal circulation, usually degenerate without causing any symptoms.

The number of syncytial knots increased in toxemia of pregnancy. These changes may be due to the occlusion or narrowing of the uteroplacental vasculature leads to placental ischemia (Soma H et al 1982). The placenta of hypertensive pregnancies (20/30 cases) showed excess syncytial knot counts indicating the placental hypoxia (Pasricha Navbis et al 2012).Aparna Narasimha et al 2011 observed increased syncytial knots in 90.4% cases in toxemia of pregnancies. Our results showed increased number of syncytial knots in preeclamptic placenta compared to normal.

Burrma et al (2013) found that syncytial knots are the principal site of expression of the antiangiogenic factors sflt-1. In addition to that they report significantly more placenta-derived syncytial aggregates in the autopsied lungs obtained from women with preeclampsia, and these aggregates still contained the antiangiogenic factor sflt-1 after their entrapment in the maternal lungs.

Augustine rajakumar et al (2012) showed that placentas from preeclamptic women have more syncytial knots that are more heavily loaded with sflt-1 protein compared with those from normal pregnancies. They concluded that detachment of syncytial knots from the placenta results in free, transcriptionally active syncytial aggregates that express sflt-1 protein.

Therefore, the increased syncytial knots are the major source of excess sflt-1 in the maternal circulation of preeclampsia.

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