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AN IMMUNOHISTOCHEMICAL STUDY OF THE SYNCYTIAL KNOTS IN THE PREECLAMPTIC PLACENTAS

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Abstract

The successful pregnancy outcome is well coordinated with the perfect placentation that requires normal trophoblastic functioning. The syncytial knots are the end products of trophoblastic life cycle which includes continuous process of apoptosis and these knots may reflect the rate of apoptosis in placental tissue. Preeclampsia or pregnancy induced hypertension has been linked with altered trophoblastic apoptosis. Our study aims for calculating and comparing the trophoblastic and syncytial knot apoptotic rates between normal and preeclamptic placentas by using M30 immunostaining. The results of our study shows increased rate of trophoblastic apoptosis and syncytial knots formation in preecamptic placentas as compared to normal placentas. The syncytial knots were higher in peripheral regions of placentas. There was a positive correlation between trophoblastic apoptotic index and number of syncytial knots in the preeclamptic placenta.

INTRODUCTION

The growing fetus requires a well formed and well anchored placenta for its normal development. Placenta is the zone structured by the intimate contact between fetal trophoblast and maternal deciduas.[1] The perfect placentation requires a well organised trophoblastic invasion of maternal endometrium as well of vascular endothelium.[2] The mechanism of invasion process requires apoptotic pathway.[3] During placenta formation, the trophoblasts differentiate and form a double layered covering around the fetus, outer being the multinucleated syncytiotrophoblastic layer, whereas inner is the mononucleated cytotrophoblastic stem cells.[4] Both the layers are vital as all the cells divide and ultimately undergo cell demise or apoptosis. The initiator phase of apoptotic cascade occurs in the cytotrophoblasts cells whereas execution phase proceeds in the syncytiotrophoblastic cells.[5]

The syncytiotrophoblastic layer plays a major role throughout pregnancy as it is the site of various placental functions which includes nutrient exchange and synthesis of various hormones like human chorionic gonadotropin, human placental lactogen required for fetal growth and development.[6] Towards the end of the trophoblastic apoptosis, old apoptotic nuclei accumulate and form membrane sealed bodies termed as syncytial knots corresponding to apoptotic bodies of the apoptotic pathway.[7] Finally, these knots are extruded from syncytiotrophoblast layer into maternal circulation. These get trapped into lung capillaries and phagocytosed by activated cells such as macrophages.[8]

The liberated knots are also known as syncytiotrophoblast membrane microparticles (STBM). As these knots are membrane sealed bodies, they prevent an inflammatory response by maternal blood vessels and organs. Apart from syncytial knots, syncytial sprouts and endocytic vesicles or exosomes are also liberated from the cell membranes.[9] As pregnancy advances, the trophoblastic apoptosis increases with progressive trophoblastic invasion and implantation.[10] The altered rate of apoptosis has been linked with various diseases and preeclampsia or pregnancy induced hypertension is one of

them.[11] The pathogenetic mechanism proposed behind this disorder is placental ischemia and hypoxia. The state of placental hypoxia may affect the trophoblast functioning and rate of trophoblastic turnover. The normal differential placental perfusion leads to central areas more oxygenated as compared to peripheral areas.[12] In the present study, we propose to compare the differential rate of apoptosis in central and peripheral areas of the placenta (by estimating the number of syncytial knots formed in these areas, calculating and correlating the rate of trophoblastic apoptosis with the rate of syncytial knot formation).

The apoptotic cells have been identified by M30 immunostaining as it identifies the cytokeratin breakdown products formed during early stages of apoptosis process in the cells of epithelial origin in contrast to Terminal deoxynucleotidyl transferase dUTP nick end labeling assay by which DNA fragmentation occurring late in apoptosis pathway can be identified in the apoptotic as well as necrotic cells.[13, 14, 15]

The study of apoptotic process in preeclamptic placentas can be a useful tool in understanding of pathogenetic changes occurring as a result of disordered trophoblastic apoptosis. Further molecular analysis of these changes can build up the unconfirmed pathogenetic mechanisms behind this disease.

MATERIALS & METHOD

Sample Collection*:* A total of 40 placentas were collected from the labour room out of which 20 were from normotensive nonproteinuric pregnant women and 20 were from preeclamptic pregnant women with gestational ages of 37-42weeks. The protocol of the study was approved by the ethics committee and consent was obtained from each individual for the study group. Patients with chorioamnionitis, chronic hypertension, pregestational diabetes, renal disease, cardiac disease, active asthma, thyroid disease and pre-existing seizure disorder were excluded from this study.

The samples were fixed in 10% formaldehyde and processed for M30 immunostaining. The techniques were standardised according to ambient conditions of our laboratory. The tissue sections were deparaffinized and rehydrated according to standard protocols.

For M30 immunostaining, all subsequent steps were carried out at room temperature in a humidified chamber. The peroxidase activity was blocked by using 3% H_2O_2 prepared in methanol for 10 minutes. The specimens were then incubated in 1-3 drops of serum block for 2 hours to prevent non-specific binding to collagen and connective tissue. The specimens were incubated for 24 hrs with M30 antibody (1:50) followed by 1-3 drops of biotinylated secondary antibody (anti mouse IgG) for 30 minutes. The specimens were thereafter incubated in 1-3 drops of HRP-streptavidin complex for 30 minutes. The slides were then counterstained with hematoxylin and dehydrated by passing through ascending grades of ethanol and slides were mounted with DPX and were observed under light microscope. The negative tissue control included eliminating the primary antiserum and replacing species-specific antiserum with normal horse serum. Sections of colon adenocarcinoma served as positive control. All 40 samples of placenta were analyzed for expression of cytokeratin neoepitope in the apoptotic cells using M30 antibody staining in serially sectioned slides. Trophoblastic cells and syncytial knots with the brown cytoplasmic stain were considered M30 immunostaining positive. The comparison between apoptotic indices (trophoblastic and syncytial knot) in normal and preeclamptic placentas was done. The correlation was done between the trophoblastic index and syncytial knot index in preeclamptic placentas. M30 apoptotic index (Total number of apoptotic cells per total number of cells multiplied by 100) was calculated for trophoblastic cells and syncytial knots. The syncytial knot apoptotic index was calculated separately for central and peripheral regions of placenta.

Statistical Analysis

Statistical analysis was carried out using Stata 9.0 / Data analysis software (college Station, Texas, USA) by using nonparametric tests. Data were presented as mean±SD or median (min-max). Wilcoxon signed rank test, Wilcoxon rank sum test (Mann whitney test) and student t test were used. The correlation was calculated using Pearson's correlation coefficient. The p value less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

In our study, the M30 immunostaining revealed higher trophoblastic apoptotic index in preeclamptic placentas (92±6.3) as compared to normal placentas (61.9±14.4) (table 1). Among the normal group, the syncytial knot index was higher in peripheral zone [25(0-40)] as compared in the central zone [0(0-25)], (p value-0.0005). Similarly, in the preeclamptic group also, we found higher syncytial knot index in the peripheral zone [(70.8(25-100)] as compared to central zone [(14.55(0-33.3)], (p value-0.0001). On comparing the syncytial knot index between two groups, the preeclamptic placentas showed higher indices in both the central (p value-0.03) as well as peripheral zones (p value-0.001) (tables 2, Figure. I). we found a positive correlation between trophoblastic index and syncytial knot index in preeclamptic placentas (coefficient 0.75, p value-0.001), (scatter diagram 1).

Discussion

The pathogenesis of preeclampsia has been the target of intense scrutiny as various theories have been proposed for this multisystem disorder. The diverse manifestations of preeclampsia including altered vascular reactivity, thrombus formation and pathological changes in various organs are due to maternal endothelial dysfunction caused by the release of multiple placental factors into maternal circulation like sflt-1, soluble endoglins and various syncytiotrohoblast products like syncytial knots, sprouts and exosomes.[16] It is also well recognized that the abnormal placentation and endothelial dysfunction are the main events occurring in this disorder and the main culprit behind these events may be the abnormal trophoblastic apoptosis. Our study shows that the trophoblastic apoptosis is exaggerated in preeclamptic placentas which causes increased syncytial knots formation in the peripheral zones of these placentas as these areas are less oxygenated and so, are more prone for hypoxic changes. The state of hypoxia leads to the stimulation of apoptotic pathway, thereby increasing trophoblastic cell turnover process.[17] The syncytial knotting represent a way of extrusion of unwanted aged nuclei. In preeclampsia, exaggerated trophoblastic apoptotic pathway can either take aponecrotic route or necrotic route of

extrusion of apoptotic material into the maternal circulation.[18]

The excessive necrosed syncytiotrophoblast micro particles interact with immune cells and endothelial cells leading to liberation of various inflammatory cytokines causing systemic maternal vascular inflammation and maternal syndrome.[19]

Being the parts of normal life cycle of trophoblastic cells, the knots can be affected by the altered rates of trophoblastic demise thereby reflecting the state of functioning of these cells in the placenta during implantation process. The understanding of molecular basis of syncytial knot formation is required which can be further helpful in establishing the pathophysiology of preeclampsia.

Table 1

Apoptotic Index (Percentage of apoptotic cells) of trophoblastic cells with M30 immunostaining in the placentas of preeclamptic and control groups

	Apoptotic index [Mean±SD (range)]		p value
Study Groups / **Cell Used for Counting**	**Preeclampsia (n=20)**	**Control (n= 20)**	
1. Trophoblastic cells	92±6.3(76.3-99)	61.9±14.4(37.3-87.9)	0.0001

n = number of subjects.

Data is presented in mean±SD (range).

Student t test for independent sample

$p < 0.05$, statistically significant

Table 2

Comparison of syncytical knot index between different zones of placentas of preeclamptic and normal group

	Syncytial Knot index Median[min-max]		p value
Zones / Study groups	**Central zone (n= 20)**	**Peripheral zone (n=20)**	
1.**Preeclamptic**	14.55(0-33.3)	70.8(25-100)	0.0001
2. Normal	0(0-25)	25(0-40)	0.0005
p value	0.03	0.001	

n = number of subjects.

Data is presented in median (min-max).

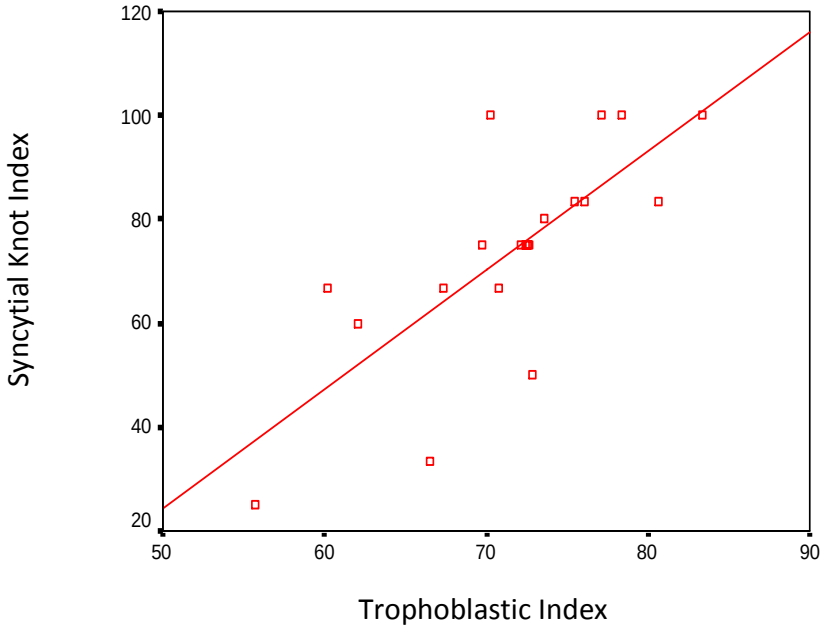
Wilcoxon signed rank test for comparison within the same group

Wilcoxon rank sum test for comparison between two groups.

p< 0.05, statistically significant

Scatter Diagram 1

Correlation of Trophoblastic index (%) with Syncytial knot index (%)



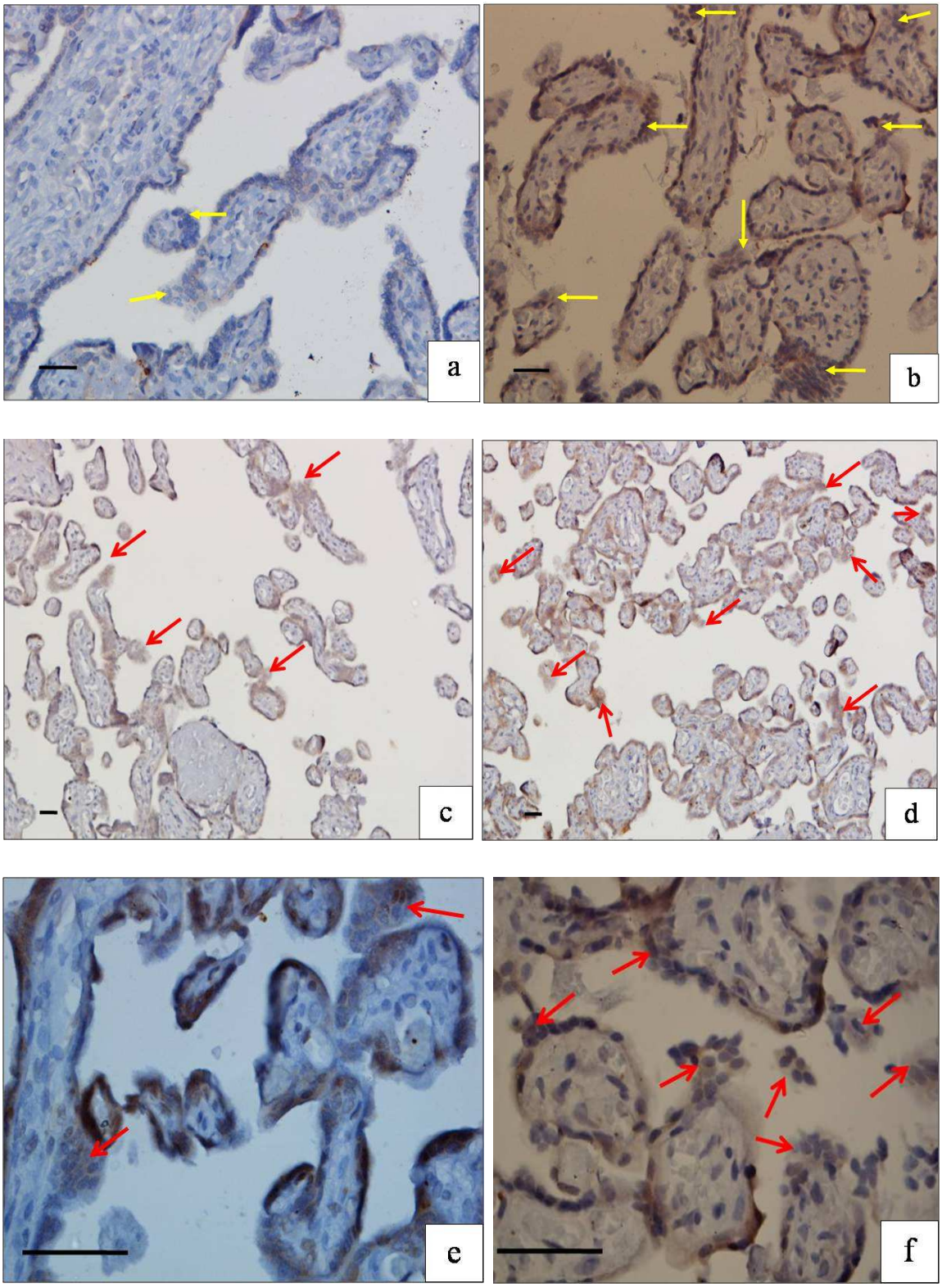


Figure 1. Photomicrograph to show the syncytial knots in the normal and preeclamptic placentas

Figure 1: Photomicrograph to show the syncytial knots in the normal and preeclamptic placentas

a. (a) Normal placenta-central zone, showing M30 positive (brown stained) syncytial knots (Yellow arrows)
b. (b) Normal placenta-peripheral zone, showing M30 positive (brown stained) syncytial knots (Yellow arrows)
c. (c, e.) Preeclamptic placenta-central zone, showing M30 positive (brown stained) syncytial knots (red arrows)
d. (d, f) Preeclamptic placenta-peripheral zone, showing M30 positive (brown stained) syncytial knots (red arrows)

Scale bar 100µm.

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