Research Article

Age Related Changes in Human Placental Oxidative Stress and its Effect on Syncytial Apoptosis (Syncytial Knots Formation)

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ABSTRACT

Oxidative stress represents an imbalance between reactive oxygen species (ROS) and cellular mechanisms for detoxifying the reactive intermediates or repairing the resulting damage, in this study oxidative stress was evaluated by anti-Mda antibodies that directed against malondialdhyde (Mda) in different placental regions (cord insertion, central, and peripheral) in different age groups (preterm, term, and postdate), and correlated with syncytial apoptosis expressed as syncytial knots count that stained with Tunel stain. A significant variation was found in anti-Mda antibody among age groups in all placental regions and syncytial knots count in peripheral region only at p≤0.05, with a highly significant positive correlation between syncytial knot counts and oxidative stress in peripheral region at termat r.0.811, and significant negative correlation at r.0.003 in postdate group in peripheral region. We conclude that oxidative stress play a major role in syncytial apoptosis as formation of syncytial knots that later on detached and liberated into the maternal circulation, this effect is more pronounced in the peripheral region of the placenta due to its distance from umbilical cord blood supply.

Keywords: Anti-MDA, Apoptosis, Oxidative stress.

INTRODUCTION

Malondialdhyde (Mda) is a naturally occurring endogenous product of lipid peroxidation that involved in covalent binding to proteins, RNA, and DNA.[1] and makes MDA-DNA adducts suitable biomarkers of endogenous DNA damage.[2] Apoptosis is important process that enable normal cells to respond to damaging stimuli as ROS, which alter DNA structure, by removing damaged cells from the organism.[3] The nuclei 'aged' within the syncytium, these 'aged' nuclei are aggregated together into syncytial knots.[4] Tunnel stain (Terminal deoxynucleotidyl transferase dUTP nick end labeling) is a method used for detecting DNA fragmentation by labeling the terminal end of nucleic acids.[5]

MATERIALS AND METHODS

A sample of 25 normal human placenta were collected from Al-Kadhimiya teaching hospital/ Obstetric and Gynecological department, delivered by C- sections, mother age ranged from 18-35 years old, and gravidity ranged from G1 to G3.The sample was divided into three groups:preterm placenta (gestational age less than 37 weeks), term placenta (gestational age from 38 - 40 weeks), and post date placenta (gestational age more than 40 weeks). Three different regions were selected from each age group: cord insertion region (at the base of umbilical cord), central region (at the center of the placenta), and peripheral region (2 cm from placental margin). Tissues were fixed in 10% neutral buffered formalin and processed for paraffin blocks.[6] Anti-Mda provided by abcam (no.ab6463), used with IHC detection kit HRP/DAB from abcam (ab80436). Slide were

deparafinized, rehydrated, hydrogen peroxide block applied, (Heat induced antigen retrieval) was performed by placing slides in (sodium citrate buffer, PH: 6), and incubated for 3 minutes in an autoclave at 120°C. Protein block done, and primary antibody was applied at 1/500 dilution and incubated at room temperature for one hour, then at 4°C overnight. Mouse specifying reagent un - conjugated was applied, Goat anti-rabbit HRP conjugate was applied and incubated for 15 minutes at room temperature. (20µl of DAB plus chromogen) was added to (1 ml of DAB plus substrate), counter stained with Harris Haematoxylin. Slides were evaluated by Aperio scope image analysis software (positive pixel count algorithm, version 9). Tunel stain was done by (ab66108) kit provided by abcam, TUNEL-based detection kit utilizes Terminal deoxyribonucleotidyl Transferase (TdT) to catalyze incorporation of fluorescein -12-dUTP at the free 3'-hydroxyl ends of the fragmented DNA. The fluorescin-labeled DNA can then be observed by the fluorescent microscope, slides were deparafinized, rehydrated, permeabilized by protinase K application, equilibrated and stained, apoptotic cells stained with (fluorescent yellow color) were evaluated in syncytial knots only and calculated in each age group in different regions at high power field (40x). Statistical analysis done using (SSPS version 18) , data were expressed as mean ± SD, ANOVA-single factor analysis used to compare among test groups in the selected regions, at P≤ 0.05 that denoted as statistically significant. Tukey´s test referred as HSD (honestly significant difference) used as a multiple comparison test that make a use of a single value against which all differences in means are compared.[7] Correlation estimation between Tunel and anti-Mda was done in

different placental regions P ≤ 0.05 was selected as the level of significance.

RESULTS

Anti-Mda appeared as a brown pigmentation in syncytiotrophoblast, and syncitial knots, in cord insertion region high value was recorded in preterm group with mean (68674.9 ± 3649.22**)** figure 1A with a significant variation among age groups in all placental regions at p≤ 0.05. Areas with fibrin deposition of types, perivillous and matrix (subtrophoblastic) fibrin were not stained for anti-Mda, but the overlying syncytium stained specially at sites of syncitial knots. In central region the highest value of anti-Mda was recorded in postdate group (111679.9 ± 5489) with a significant variation among groups at p ≤ 0.05. (HSD: 90.88). (Table 1), figure (1B) In peripheral region highest value of anti-Mda seen in postdate group (91505.2 ± 6812.35**)** with a significant variation among groups at p≤ 0.05, and (HSD: 76.78). Table (1), figure (1C).

Table 1: Mean number of strong positive pixel of anti-Mda antibody in different placental region in different age groups, and its statistical significance

Regions	Cord insertion	Central	Peripheral
Groups	Mean± SD	Mean± SD	Mean± SD
Preterm	68674.9 ± 3649.22	27317.5 ±6919.	34813.9 ± 5491.08
Term	37705.3 ± 9429	33971.7 ± 4125.82	36523.9 ± 9083.18
Postdate	27131.2 ± 38433.77	111679.9 ± 5489.	91505.2 ± 6812.35
p-value (≤0.05)	*0.037438 (significant)	*0.010989 (significant)	*0.004573 (significant)
HSD	54.6	90.88	76.78

*P value ≤ 0.05 considered statically significant

Table 2: Mean number of syncitial knots count in different placental regions in different age groups, with statistical significance.

Regions	Cord insertion	Central	Peripheral
Groups	Mean number of syncitial knots ± SD	Mean number of syncitial knots± SD	Mean number of syncitial knots± SD
Preterm	2.8± 1.4	4.2± 2	3± 1.3
Term	4.1 ± 1.5	3.5 ± 1.3	4.6 ± 1.3
Postdate	3.3 ± 1.5	5.1 ± 1.7	6.7 ± 1.3
P value ≤ 0.05	0.13881 (non significant)	0.14 (non significant)	*0.000000659 (significant)
HSD (Tukey´s test)	3.7	4.53	10.499

*P value ≤ 0.05 considered statically significant.

Tunel stain for syncytial knots revealed highest value in term group in cord insertion region with mean value 4.1 ±

1.5 with a non significant variation in syncitial knot count among groups at p ≤ 0.05, HSD: 3.7. Table (2), figure (2A). In central region syncitial knots count showed highest value in postdate group 5.1 ± 1.7, analysis of variance among groups showed a non significant variation at p ≤ 0.05, HSD (4.53), Table (2), Figure (2B), in peripheral region syncitial knots count showed highest value in postdate group 6.7 ± 1.3, and analysis of variance showed a significant variation at p≤0.05, HSD: 10.499. (Table 2), Figure (2C). A highly significant positive correlation was found between syncytial knot counts and anti-mda in term placenta in peripheral region at r. 0.811, and a negative significant correlation between syncytial knot counts and anti-mdaAb in postdate placenta in peripheral region at r.0.003. Figure 3.

DISCUSSION

Increased oxidant formation and oxidative stress is considered normal during pregnancy, due to elevated generation of free radicals by placental mitochondria. [8] In this study Anti-Mda appeared within the syncytiotrophoblast and syncitial knots, due to the presence of an enzyme in syncytium that is involved in the production of oxygen radicals that play a role in oxidative stress, this is nicotinamide adenine dinucleotide phosphate (NAD[P]H) oxidase which is an important enzyme that generates superoxide radicals localized in the placenta syncitialmicrovillous membrane, which may play a role in placental lipid peroxidation by generating increased amounts of the superoxide radical. [9, 10] Regional variations in the intensity of anti-Mda in different placental regions was evident by microscopic examination that confirmed by aperio scope image analysis software , being significantly high in postdate group in central and peripheral regions compared to term group,this due to increased production of free radicals and reduction in antioxidant effect in postdate group, as the placenta is very susceptible tissue to oxidative stress and pregnancy is a state of oxidative stress started from early pregnancy to term, this raised from increased placental mitochondrial activity and production of reactive oxygen species (ROS). [11] In cord insertion region anti-MDA showed high value in preterm group with low syncytial knot counts, this could be due to vascular lesions in cord vessels in early pregnancy that lead to creating hyperoxic environment, and due to the regulatory influence of local oxygen partial pressure on the trophoblast turnover, where the low PO2 gradient the cytotrophoblast proliferation is stimulated, while in zones of higher PO2gradient as in cord insertion, the poorly proliferating cytotrophoblast fuses and finally disappeared without formation of syncytial knots. [12] Loss of villous cytotrophoblast normally accompanies hyperoxia [13], furthermore an involution of the villous cytotrophoblast and, finally, degenerative/apoptotic changes of the syncytiotrophoblast was observed in tissue hyperoxia. [14] In peripheral region a significant variation in the syncitial knot count, with high value in postdate group could be part of normal turnover of trophoblastic tissue that

occurred during pregnancy, an apparent increase in apoptosis in postdate placenta was found compared to that in preterm, midterm placenta, this reflect aging of the placenta or parturition associated biological change.[15] Apoptotic changes in the peripheral regions most probably is due to oxidative stress effect as its value is high in postdate group, as a significant positive correlation was found between apoptotic syncitial knot count and anti-Mda at r.0.811, p. 0.0001 in term group, this agreed with who suggested that syncitial knots formation is part of normal turnover of trophoblastic tissue of human placenta, and its amount changed throughout normal pregnancy, being lowest in the first trimester, increased in the third, and markedly accelerating beyond 40 weeks gestation, since part of the turnover of villous trophoblast includes proliferation, differentiation of cytotrophoblast, syncitial fusion of cytotrophoblast with the overlying syncytiotrophoblast, differentiation in the syncytiotrophoblast, and finally extrusion of apoptotic material into the maternal circulation.[16,17] Hypoxic conditions in the peripheral region could induce oxidative stress and this affect syncitial fusion, this increase in syncitial knot count in hypoxic condition, in presence of ROS, and chronic hypoxia or alternate periods of hypoxia/re-oxygenation within intervillous space is expected to trigger tissue oxidative stress and increase placental apoptosis, and as true syncytial knots reflect a characteristic feature of syncytiotrophoblast apoptosis.[18] The effect of hypoxia has been demonstrated on syncitial fusion in cell line, and found that under hypoxia syncytin mRNA levels decreased, and decrease syncitial fusion.[19,20] So the increment of syncitial knot counts in peripheral region could reflect aging of the placenta in peripheral region and an epigenetic modifications underlie these changes in chromatin appearance, that lead to syncitial nuclei continue to accumulate until postdate, and the apoptotic cascade that is initiated in the villous cytotrophoblast, which in turn promotes syncitial fusion, and completion of the apoptotic cascade takes place in and around syncitial sprouts, providing further evidence that these are the sites of trophoblast shedding into the maternal circulation.[21] This suggested that apoptosis has a central role in the villous trophoblast turnover, and alterations to placental function by external factors such as hypoxia and reactive oxygen species can lead to significant increases in placental apoptosis. On the other hand we found that a significant negative correlation between Mda and syncitial knot counts in postdate group in peripheral region at r.0.636, p.0.003, this could be due to that in this region excess oxidative stress could lead to syncitial loss through formation of syncitial knot that later on detached into intervillous space, and lead to reduction in their counts leaving thin layer of syncytium. As a consequence of progressive apoptotic degradation of syncytiotrophoblast cytoskeleton, the aging nuclei were focally aggregated, forming apoptotic knots, which shortly were pinched off from the syncitial surface and deported into maternal circulation.[22]

Figure 1: Anti-Mda antibody in human placental trophoblast appeared as brown deposition within syncytium and syncytial knots in (A) preterm cord insertion region, (B) postdate in central region, (C) postdate in peripheral region.40x
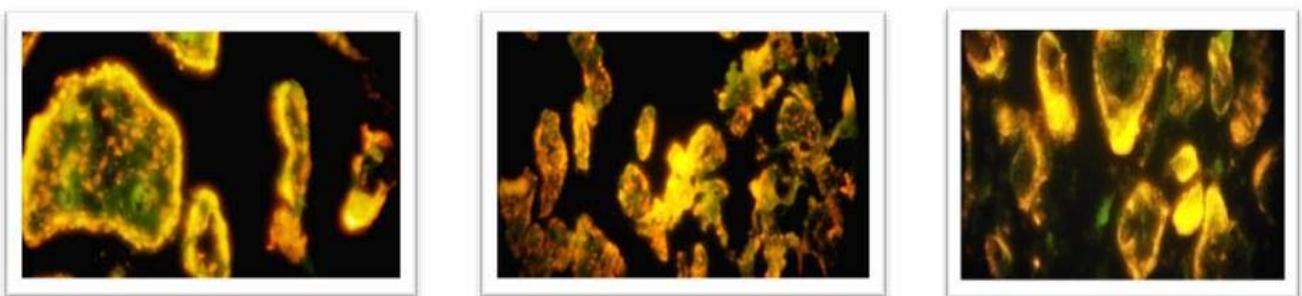

Figure 2: Tunel stain in human placental trophoblast appeared as fluorescent yellow color at syncytial knots in (A) preterm cord insertion region, (B) postdate in central region, (C) postdate in peripheral region.40x
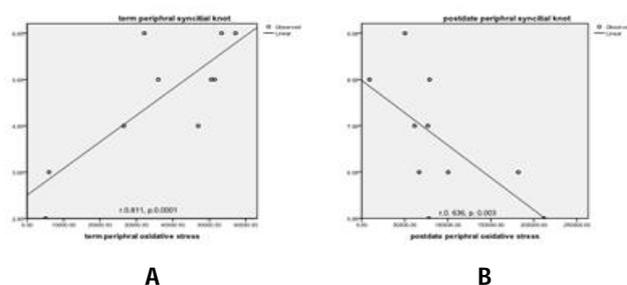
| A | B |

Figure (3/A): Positive significant correlation between syncitial knot and MDA in term group, peripheral region. (3/B): Negative significant correlation in postdate peripheral region.

CONCLUSION

Oxidative stress has a pronounced effect on syncytial aging that differ in different placental regions, where in cord insertion oxidative stress mostly due to tissue hyperoxia due to umbilical vessel lesions that triggered syncytial apoptosis without increase in syncytial knot counts, while in peripheral region oxidative stress mostly in form of hypoxia due to their distance from blood supply increased syncytial aging by increasing syncytial knot counts.

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Source of Support: Nil, **Conflict of Interest:** None.