



PLACENTAL FACTORS IMPLICATED IN INTRAUTERINE GROWTH RESTRICTION

Krishna Rao Gurugubelli and Vishnu Bhat Ballambattu*

Department of Neonatology, Jawaharlal Institute of Post Graduate Medical Education and Research (JIPMER),
Puducherry-605006, India.

***Corresponding Author: Dr. Vishnu Bhat Ballambattu**

Department of Neonatology, Jawaharlal Institute of Post Graduate Medical Education and Research (JIPMER), Puducherry-605006, India.

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ABSTRACT

Intrauterine growth restriction (IUGR) is defined as the failure of the fetus to reach its genetic growth potential. It is one of the most frequent obstetrics problem in developing countries affecting approximately 30 million infants every year. The placenta is least understood human organ, which plays a crucial role in normal fetal growth. Any disturbance in placenta development leads to pregnancy complications like IUGR and until now no therapy interventions are available for this pathology. This review emphasises on the possible mechanisms in placenta like differential expression of proteins, deregulated pathways, DNA methylation changes that contribute to the pathogenesis of IUGR and also explains the various possible treatment modalities for placental repair and development.

KEYWORDS: Intrauterine Growth Restriction, DNA Methylation, Placenta, Infants.

INTRODUCTION

Intrauterine growth restriction (IUGR) is a condition where the growth rate of a fetus is less than the normal genetic potential at a particular gestational age. Most of the IUGR babies are small for gestational age, in which birth weight is below the 10th percentile for a particular gestational age. Its diagnosis is made based on comparing gestational age with fundal height and ultrasound examination.^[1-3] Some of the known causes of IUGR are maternal malnutrition, smoking, alcohol or drug abuse and twin pregnancies etc. but many of them share placental abnormality.^[4] Its normal development is crucial for proper fetal growth as oxygen and nutrients are supplied to the developing embryo through placenta. Altered placental transport of nutrients has been associated with increase the risk of growth restricted baby.^[5] Placental pathology affects both mother and fetus. Various Placental factors (differential expression of proteins, deregulated pathways, DNA methylation changes) are implicated in fetal growth restriction. This review emphasis on possible placental factors implicated in fetal growth restriction and also explains the possible therapeutic interventions for placental repair and development.

Structure and function of placenta: The human placenta is disc shaped and least understood organ which acts as a barrier between mother and fetus.^[6] In term human placenta consists two cell layers, such as fetal capillary endothelium and syncytiotrophoblast. These two cell layers separate the fetal and maternal circulation.

The syncytiotrophoblast consists of microvillous membrane (facing towards intervillous space) and basal membrane (directed towards fetal capillaries).^[5] A recent 3D microscopic analysis of villous of IUGR placenta showed larger branching angle at terminal positions of villi. The preterminal part of villous tree do not show a prominent tortuous architecture and vice versa in normal placenta.^[7] The stiffness and viscosity is reduced in IUGR placenta compared to normal placenta.^[8] Understanding about the placenta at ultra-structural level gives an idea about IUGR and in utero fetal development. In the course of pregnancy, placenta enlarges its surface area for exchange of gases like oxygen, carbon dioxide and metabolic molecules such as glucose, amino acids, fatty acids, electrolytes, vitamins and water. It also serves endocrine function, mainly involved in the production of peptide hormones like human chorionic gonadotrophin, human placental lactogen, human growth hormone variant and steroid hormones like oestrogen and progesterone. It is also involved in the passive immunity to the fetus in first few months of life by transferring the IgG antibody from mother to fetus.^[6]

Differential expression of proteins in placenta of IUGR fetus: Hctd1 is a protein that plays an important role in the development of placental labyrinthine zone, which functions as transfer media of nutrients and waste materials between mother and fetus. Hence mutation of Hctd1 protein results in pale placenta with prevalent hyphema and atypical structure of labyrinthine zone including poorly elaborated fetal vasculature containing

minimal fetal blood cells.^[9] Hectd1 is indispensable for improvement of placenta.

Mutation of this protein results in reduction of the number and nuclear size of trophoblastic giant cells, lowered expression of PL1, PL2 and elevated expression of proliferin and elevated immature uterine natural killer cells in the maternal decidua of placenta together contributing to the development of IUGR.^[10]

Along with gestational age, fetal gender also influences the expression of galectin variants like gal-1, gal-2, gal-3 and gal-13 in the trophoblast cells of IUGR placenta. But expression levels are different in different compartments of trophoblast.^[11] Fetal gender specific expression of tandem repeat galectins were seen in IUGR placentas, which consists of gal-4, gal-6, gal-8, gal-9 and gal-12. Among them gal-6 was only well expressed in Gal-4,8,9 were less expressed in IUGR placental trophoblast of male fetus while elevated expression of gal-9,12 in the extra villous trophoblast was noted among IUGR pregnancies with female fetus.^[12] Prenatal caffeine exposure (PCE) reduces expression of adenosine A2 receptor, which in turn decreases cyclic AMP production lowering the expression of placental CREB and leptin. PCE also inhibit placental Ob-Ra receptor expression, which causes impaired maternal leptin transportation to fetus.^[13]

Diminished activity of placental 11 β -hydroxy steroid dehydrogenase type 2 activity was observed in IUGR fetus compared to small for gestational age fetus.^[14] In IUGR placenta elevated expression of FK506-binding protein (FKBP52) was noted in the pathogenesis.^[15] In placental related IUGR there was increased chance of cardiovascular risk biomarkers like natriuretic peptide, cTnT and h-FABP in cord blood.^[16] Elevated expression of placental pleckstrin homology like domain family A member 2 (PHLDA2) was seen in in utero growth restriction in response to environmental stimuli such as maternal life style.^[17] Increased placental expression of PHLDA2 is associated with the pathogenesis of selective IUGR in monozygotic twins.^[18] Placental dysfunction in IUGR is associated with increased levels of Inhibin A, activin A, and follistatin in umbilical vein and maternal serum which can serve as novel markers for detection of IUGR.^[19] In IUGR pregnancies placental expression of epidermal growth factor was reduced compared to normal pregnancies.^[20] Due to over expression of placental endoglin [CD 105] gene vascular dysfunction occurs leading to chronic fetal hypoxia.^[21]

Placental apoptosis is one of the characteristic feature of IUGR, in which remarkable elevation of caspase-3 is seen in the villous trophoblasts.^[22] Lowered Bcl2 gene expression was observed in the IUGR associated pregnancies but there was no difference in the Bax gene expression between the IUGR and appropriate for gestational age babies.^[23] In IUGR placenta the expression of vascular endothelial growth factor

(VEGF-A) gene is increased and leads to angiogenesis in response to hypoxic environment.^[24] IUGR complicated female fetus with placental mesenchyme dysplasia is associated with increased VEGF-D expression.^[25] Placental vascularity also depends on the expression of MMP-9 and TIMP-2. In IUGR pregnancies lowered expression of MMP-9 and higher expression of TIMP-2 was observed.^[26] Prostate specific placenta antigen content was found to be higher in the preeclampsia group with IUGR.^[27] Transthyretin is a carrier for thyroxine and retinol binding protein. In pregnancies complicated with IUGR placenta deregulated transthyretin was seen.^[28] The expression levels of the pentraxin-3 (villous stroma, decidual cells and terminal villi) and TNF- α (trophoblast, vascular endothelial cells, decidual cells) were significantly increased in preeclampsia with IUGR.^[29] Placenta of ovine IUGR model showed increased concentration of osmolytes and TonEBP/NFAT5 expression and a comparable rise of AR and SLC5A3 enzymes.^[30] PIGF serves as a better biomarker in identification of placental IUGR and it is also used to demarcate placental IUGR from constitutionally small fetuses with high sensitivity and specificity.^[31] A study conducted on the pregnant mice showed that treating with dexamethasone will lead to IUGR and induce fetal developmental toxicity by disruption of glucocorticoid barrier and fetal adrenal function.^[32] Fig. 1 shows the differential expression of proteins during fetal development leading to IUGR.

Dysregulated mechanisms in placenta during IUGR

By studying human placental mesenchymal stromal cells (pMSCs), novel mechanisms in IUGR were deciphered. A study reported that decreased differentiation potential of placental mesenchymal stromal cells towards the endothelial phenotype and elevated adipogenic ability of pMSCs play a prominent role in the reprehensible cycle of defective placental development.^[33] Lowered expansion of maternal plasma volume results in reduced placental and fetal growth by placental hypoxia, increased glycogen cell aggregation in junctional zone of placenta leading to defective invasion of trophoblasts which mediates fluctuations in reconstruction of uterine arteries.^[34] Control of placental nutrient transports prominently influence the intrauterine growth and alter fetal development. Increased placental fatty acid transporters (FABP, CD36) and reduced amino acid (SNAT1, SNAT2), glucose transporters (GLUT-1, connexin-25) possibly cause intrauterine programming.^[35] In order to achieve fetal growth and development, both placenta and endometrium play a prominent role in effective nutrient transportation, which requires energy, transporters and ingrained vascular system. Defect in proteins related to nutrient transportation and vascular genesis contribute to development of IUGR.^[36] Nectin4 is a extracellular member of laminin related protein family involved in the regulation of endothelial growth in placental villi by inhibiting the human placental microvascular endothelial cells (HPEC) angiogenesis related to IUGR.^[37]

Insulin like growth factor-2 (IGF-2) plays prominent role in development of placenta and fetus. In placental trophoblast stem cells of IUGR, superior expression of microRNAs such as miR141-3p and miR-200a-3p inhibits the expression of IGF-2, leading to impaired Akt activation resulting in aberrant placental development.^[38] Pre supplementation of vitamin D3 suppress the lipopolysaccharide induced placental inflammation by stimulating vitamin D receptor signalling, which in turn suppresses the activity of nuclear factor kappa B (NF- κ B) and also inhibits the nuclear translocation of NF- κ B p65 subunit in trophoblast cells in LPS induced IUGR.^[39] Higher expression of myostatin was seen in the pregnancies complicated with IUGR and preeclampsia, which helps to support the pregnancy rather than interrupt.

This was revealed by a study in which first trimester placental explants were treated with myostatin which resulted in lowered production of Th1 pro inflammatory cytokines and higher production of Th2 anti-inflammatory cytokines.^[40] Studying the transcriptome analysis of human IUGR placenta unveils that concerned genes related to functioning of mitochondria, oxidative phosphorylation were decreased and this affected the mitochondrial electron transport chain disruption in energy production and metabolisms, in which most elicited genes are LEP, IGFBP, RBP4.^[41] Hyper methylation of the RASSF1 gene promoter was accompanied by lowered expression in placentas from pregnancies complicated with IUGR.^[42] Lowered phosphorylation of m TOR protein is associated with diminished trophoblast invasion and that PlGF signalling can equilibrate to inflate invasion of these cells by phosphorylation of p70 independent of m TOR.^[43]

Placental perfusion is essential for the exchange of nutrients and gases and waste materials between the fetal and maternal circulation. Over expression of the SK3 (Calcium activated potassium) channel alters the balance between VEGF (angiogenic) and sFlt-1 (anti angiogenic) factors which affect vascular development of placenta.^[44] Decreased placental vasculature is one of the characteristic feature of IUGR. Trophoblastic cells play an important role in the promotion of placental vasculature by autocrine and paracrine mechanisms of IGF-1 on human placental microvascular endothelial cells.^[45]

Deregulation of placental micro RNAs is a key factor in the placental associated IUGR and the decreased levels of mature miR-34a imparts elevation of SERPINA3 in placental diseases.^[46] Placental GLUT3 expression was increased in the IUGR placenta due to increased concentration of hypoxia mediating factor HIF-1 α .^[47] Vascularity of umbilical and placental vessels is maintained by the nitric oxide derived from the endothelial nitric oxide synthase activity and in this process there is a race between the eNOS and arginase-2 on L-arginine. Hence asymmetric ratio of eNOS and

arginase-2 activities significantly contributes to the fetal placental vessels of IUGR.^[48] The higher expression of Nrf-2 genes in villous cytotrophoblast and extravillous trophoblast lower the VEGF expression and lead to oxidative stress. This oxidative damage may contribute in the narrow trophoblast invasion into uteroplacental arteries of Preeclampsia and IUGR.^[49] Increased oxidative stress in IUGR placenta leads to conformational changes of ApoB lipoprotein, which results in deposition of oxidised LDL particles in IUGR placental tissues and minimize the availability of cholesterol to the fetus and also lower in fetal serum cholesterol levels.^[50]

Increased levels of mt DNA was seen in the preeclampsia with IUGR than without IUGR, which indicates an attempt to compensate for placental diseases with other features.^[51] Plac1 (Placenta specific 1) gene is a paternally imprinted X linked gene, significant in the placenta development, hence Plac1 ablation results in development of mild IUGR.^[52] A study conducted on the rodents by injecting caffeine before and during pregnancy results p53 dependent placental apoptosis due to degenerative induction of the maternal and placental renin angiotensin system activity.^[53] Intricate interactions between the insulin like growth factor axis and its imprinted genes with gestational maturity and fetal growth determine growth suppressive genes in placentas of fetus with IUGR.^[54] In pregnant rats exogenous hyper insulinemia accrue devising of endothelin-1 by serial proteolysis of large endothelin by endothelin converting enzyme-1. Its expression is stronger in the trophoblast cells of placenta, which may lead to pregnancy induced hypertension and IUGR.^[55] Over expression of IGF-2 and IGFBP-3 genes was found in the IUGR placentas and it leads to the impaired insulin and carbohydrate metabolisms.^[56]

DNA Methylation

The cellular basis of fetal programming is mediated by epigenetic mechanisms such as DNA methylation. DNA methylation predominantly occurs at cytosine in cytosine-guanine (CPG) dinucleotide and it is an essential epigenetic impression in mammals which contributes to regulation of Gene transcription. It plays crucial role in Genetic behavior of various diseases including cancer, auto immune diseases, inflammatory diseases, neurological disorders and also IUGR. Insulin growth factor (IGF 2) is a key prenatal growth factor. A typical imprinting and altered expression of IGF2 occurs from paternally inherited allele under control of DNA methylation which leads to Beckwith-wiedemann (associated with prenatal overgrowth) and silver Russell syndromes (associated with marked intra-uterine growth restriction (IUGR)).^[57] Studies have shown that increased DNA methylation at the promoter of nuclear transcription factor –Hepatic nuclear factor 4 α (HNF4A) plays a role in IUGR.^[58]

Glucocorticoids play a significant role in fetal growth and development and maturation. Expression of glucocorticoid receptors (GRs) are seen in most fetal tissues including placenta from early embryonic stages. Evidence from animal and human studies reveals that excess of glucocorticoids in utero impair fetal growth, and noticeably IUGR neonates appear to have elevated cortisol levels by methylation of first and third CpG sites of placental HSD11B2 gene promoter.^[59] Studies have reported that methylation and expression of imprinted genes (CDKN1C, PEG10, PLAGL1, SNRPN, and MEST) was similar between IUGR and control placentas, H19 gene promoter is hypo methylated and its expression was increased in placentas from pregnancy complicated with fetal growth restriction.^[60] Monozygotic monochorionic twins are severely affected by IUGR due to sharing of similar genotype and maternal environment during conception. Studying genome wide methylation analysis of MM-twins placenta revealed differential methylation in the promoter region of genes such as DECR1, ZNF300, DNAJA4, CCL28, LEPR, HSPA1A/L, GSTO1, and GNE. Among these high methylation of DECR1 and ZNF300 causes disruption in beta oxidation of fatty acids and transcriptional regulation respectively, and it significantly affects the Wnt and cadherin pathways in IUGR babies.^[61] DNA methylation at the promoters of INHA, FN1, PAPA in the term placenta was positively associated with the levels of INHA, FN1 proteins and negatively associated with PAPA protein levels in maternal serum of early pregnancy.^[62]

DNA methylation and consequent changes in placental expression of PLAGA1 may execute fetal growth and results in IUGR by deregulation of the system of genes like SLC2A4, TCF4 and PPARG1 in human placenta.^[63] Maternal environmental factors influences the outcome of a baby which is evident from a study conducted on rat models. It revealed that maternal gestational protein deficiency alters the methylation and expression pattern of genes like Wnt2, Dlk-1 of placenta and contributes to the development of IUGR.^[64] p66Shc is an adaptor protein which produce mitochondrial ROS and modifies oxidative signals into apoptosis, the lowered methylation patterns of p66Shc promoter significance in endothelial dysfunction and cardiovascular diseases in IUGR babies.^[65] Syncytin-1 is an enveloped gene of human endogenous retrovirus ERVW-1 and is one of the crucial gene involved in the cell fusion. Due to hyper methylation of ERVW-1 promoter syncytin-1 expression is reduced in placentas related to IUGR.^[66] Fetal derived hyper methylated RASSF1A in maternal plasma is considered as a latent biomarker to initiate placenta associated pregnancy complications like IUGR.^[67]

Therapeutic interventions for placental repair and development: The administration of sildenafil citrate to IUGR rabbit model improved the development of placenta by increasing vascularity and vessel

hypertrophy in the decidua and also showed increased resistance and pulsatility indices at the middle cerebral artery.^[68] Placental insufficiency necessitates development of IUGR which is a leading cause for development of adult onset metabolic diseases. Presently no remedies available for treatment of IUGR with placental insufficiency. Nano fabric transport system, which consists of HPMA-bpDMAEMA copolymer accomplish to hIGF-1 plasmid DNA under the regulation of trophoblast specific promoters (CYP 19a or PLAC 1) bring expression of IGF-1, which is competent in maintaining normal fetal growth in IUGR mouse model.^[69] It is also carried out by adenoviral mediated gene transfer, which rectify placental insufficiency by increasing the expression of glucose transporters in both human and mouse placenta.^[70] Supplementation of Docosahexaenoic acid inhibits placental apoptosis by lowering the activity of caspase 3.^[71] Placental insufficiency and consequent IUGR intensify the chance of cerebral palsy and other developmental disabilities. This can be prevented by maternal dietary supplementation of broccoli sprout, an affluent source of isothiocyanates observed in IUGR rat model.^[72] Vector mediated gene therapy by adenoviral mediated placental gene transfer of IGF-1, increases the placental glucose transport mechanisms in mouse model of IUGR and human trophoblast cells.^[70] Embryonic exposure of lithium, homocystine, alcohol in mouse contributes to development of IUGR by defective expression of nonmuscle myosin-11 (NMM-11) in the mouse placenta, but early pregnancy supplementation of folate and myoinositol can reconstruct NMM-11 expression and facilitate normal placenta development.^[73] A study conducted on the IUGR ovine fetus by injecting intra amniotic IGF-1 results in increased fetal growth by up regulating the expression of mRNAs for placental transporters of various types of amino acids through the increased activation of the mTOR protein.^[74] Prenatal smoking and alcohol consumption causes IUGR due to irregular antioxidative and transport functions of placenta. A study conducted by treating the IUGR (induced by prenatal alcohol/tobacco exposure) rats with sodium ferulate, reverses the IUGR by enhancing the antioxidative property and P-glycoprotein expression of the placenta.^[75] Oxidative stress creates a destructive effect to functionality of placental mitochondria. In vitro glutamate supplementation to mitochondria of human term placenta, regulates the level of lipid peroxidation in pregnancies and prevents the free radical complications such as IUGR, preeclampsia, which can affect the health of both mother and fetus.^[76] No effective therapies still exist for IUGR. Some of the attempts that researchers performed during the last years in order to find effective interventions for IUGR, but mechanisms underlying the effects and the interactions of these interventions with the complex human machinery during pregnancy are still not sufficiently elucidated.

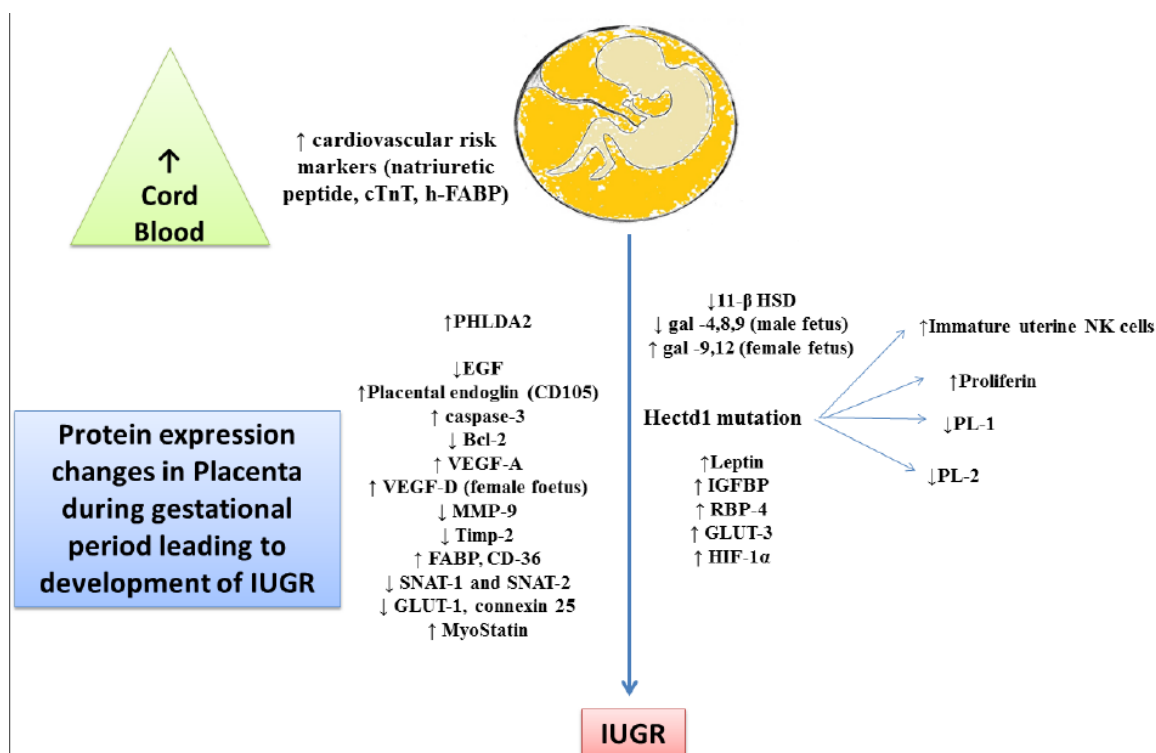


Figure. 1. shows the differential expression of proteins in placenta during gestational period leading to IUGR development.

CONCLUSION

IUGR is a very serious disorder which delays the growth and puts the baby at risk of certain health problems during pregnancy, delivery and afterbirth. It has many possible causes like problem with placenta, placental insufficiency. Several mechanisms such as differential expression of proteins, dysregulated mechanisms of placenta and epigenetic mechanisms such as methylation may also play a crucial role in pathophysiology of IUGR. Understanding these mechanisms may help design effective strategies for early prevention and treatment of IUGR.

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CONFLICT OF INTEREST

Authors have no conflict of interest to declare.

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This is a review article.

CONTRIBUTION OF AUTHORS

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