Review Paper:

A Decade Review: Preterm Labor and Homocysteine

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Abstract

Preterm labor is one of the uprising cause for maternal, foetal and neonatal morbidity and mortality health-related problem in the world. Preterm labor is defined as birth occurring before 37 weeks of gestation. Several biological and environmental factors have been playing an important role in the occurrence of preterm labour. One of the cases reported in past decades is elevated homocysteine level which had shown an association with preterm labour. Homocysteine is non-protein associated amino acid which is formed with the homogenization of methionine to cysteine or it gets remethylated.

Elevated circulating levels of homocysteine are generally known as an unrestrained risk factor for pregnancy and other disorders e.g. PTL. The levels of homocysteine can be increased by a defect in the formation of methionine, which results in gene mutation of homocysteine or folate metabolisms. In this study, we have attempted to throw light on the importance of metabolic/ biochemical pathway and genetics of homocysteine which can help play cornerstone in research.

Keywords: Preterm labour, period of gestation, homocysteine, risk factors.

Introduction

The period of gestation is one of the most important predictors of health and survival for an infant as well as for the mother that cumulates 62% of neonatal death[14]. The gestation period has been affected by several etiopathological factors such as premature rupture of membrane, preeclampsia, abortion, preterm labour, recurrent spontaneous miscarriage etc[28,35,38]. WHO (World Health Organisation) defines Preterm labor (PTL) as "birth occurring before 37 weeks of gestation". Further, WHO has classified preterm into three categories i.e. extremelypreterm (<28 weeks of gestation), very preterm (28-38 weeks of gestation), moderate/late preterm (32- <37 weeks of gestation)[5].

In several cases, detailed reports or mechanisms for the preterm labor are uncertain, but in recent studies, it has been observed that other than environmental and biological factors[23,41], biochemical changes in maternal metabolism show a positive association with PTL, among which alteration in level of homocysteine has attracted many researchers[36]. Homocysteine is an amino acid which does not form a protein. It is involved in the transsulfuration pathway and remethylation pathway, which plays a key role in preventing circulatory problems in humans[6,34].

During past decades, researchers had observed an increase in the serum level of homocysteine in both normal and pregnant women. In addition to its association with PTL, altered homocysteine in both pregnant and non-pregnant individual has been claimed to be associated with several pathologies like diabetesmellitus, hypertension etc.[49,27]

Risk Factors

Many risk factors have been contributed to escalating the risk of PTL. These factors can be broadly classified as environmental, biological sociological factors.

Environmental risk factors: Increasing pollutants and artificial chemical (Xenobiotic) are emerging as risk factors for PTL e.g. chemical pesticides, antibiotics, biomedical waste etc. Moreover many other pollutants, which are non-oxidative like sulphur dioxide that emerge from burning of minerals, ores, carbon compounds, those are used or released from daily activities (e.g. Chlorofluorocarbons from refrigerator, carbon dioxide from burning of fuel and deforestation, petrochemical agents such as glues, dry-cleaning products etc.) and methane gas which happens to get released out by burning of fuel from petrochemical industries as well as vehicles, these environmental factors have also been shown as risk factors for PTL[3,4,24,33,41,44].

Sociological risk factors: Some sociological factors like a race of an individual have also been encountered as a risk factor i.e. marriage between interracial couples e.g. women getting married to African American men. Age of the couple is proportional to healthy reproductive life e.g. mothers between the age group less than 22 and above 32 years are more prone to PTL[20]. Studies have shown that women with multiple partners or unmarried due to social security have led to PTL[47]. Low-income families or poor income with a large number of people in the families have resulted in PTL due to improper care or deficiencies[19]. In some cases, it was observed that habits such as alcohol consumption, smoking etc. have a deleterious effect on mother as well as on foetus resulting in death, intrauterine growth retardation (IUGR), intrauterine foetal death (IUFD)or PTL[2,8,12,42].

Biological risk factors: Many microbes like bacteria causing vaginosis trichomoniasis, tuberculosis;[32,39] viruses

that cause sexually transmitted disease such as gonorrhoea, syphilis have a role in causing PTL[26]. Other than that, parasitic infections like malaria, worm infestation etc.[9,25] have led to PTL. Researchers have experienced that several blood disorders like haemophilia, anaemia and increased maternal blood problem (e.g. preeclampsia) have played an important role in PTL either by fluctuating or decreasing requirements in foetal circulation[37,40].

Moreover, some reproductive problems such as rupture of the uterus, vaginal bleeding which have occurred due to fall or stress; improper implantation like placenta previa, or artificial insemination/ *in vitro* fertilization have also contributed in PTL[11,18,45,48]. Studies have proven that positive familial history, genetic factors like gene mutation, single nucleotide polymorphisms (SNP), immunological polymorphisms linked with cyclin-dependent kinase (CDK) or interleukins have shown association with PTL. Studies on metabolic-related mutations or polymorphisms in genes have shown lanosterol 14α-demethylase (CYP51A1), 7-dehydrocholesterol reductase (DHCR7), 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGCR), glutathione S-transferase µ1 (GSTM1), paraoxonase/arylesterase 1 (PON1) etc. identified as a risk factor in PTL.[41] Some studies reported that escalated serum homocysteine level dysregulates folate pathway affecting PTL, along with few of its known enzyme causing single nucleotide (SNP) e.g. MTHFR, SHMT, TYMS etc.[10,31,46].

The genetic basis of homocysteine and PTL: During transmethylation, homocysteine gets involved with folate resulting in the production of MTHFR (methylenetetrahydrofolate reductase), thymidylate synthase (TYMS) and serine hydroxymethyltransferase (SHMT) genes. This gene could be responsible for causing inborn error metabolisms in the foetus or pregnancy-related problems in mothers. Hyperhomocysteinemia is caused due to defect in remethylation or transsulfuration process resulting in disturbances of MTHFR enzyme deficiencies leading to MTHFR gene mutation. The malfunction of N_5-methyltetrahydrofolate takes place due to which rapid cell division takes place, causing many problems like PTL, anaemia etc.[6,31]

Similarly, another metabolic enzyme which involved remethylation is TYMS, this enzyme is used in the regulation of DNA synthesis. N_5, N_{10} methyl donor is used for remethylation process. This metabolism causes many other gestational problems such as abruption placenta, recurrent pregnancy loss, preeclampsia, IUGR etc.[6,31] VitB-6 dependent enzyme is serine hydroxymethyltransferase (SHMT) and it acts in the remethylation of THF converting to N_5–methylterahydrofolate (MTHF) into N_5, N_{10} - methylterahydrofolate; disturbances in this part of folate pathway lead to PTL[6,10].

Homocysteine involvement in folate metabolisms by transmethylation process had shown strong relation and proven that disturbance in this could lead to several other gene mutations too like betaine-homocysteine methyltransferase (*BHMT*), methionine synthase reductase (*MTRR)*, reduced folate carrier (*RFC1*) and methionine synthase (*MTR*)[7]. Fewer studies, which have been conducted on association of homocysteine and PTL in the last decade are enlisted (Table:2).

Nutritional or Biochemical: Cumulative pieces of evidence support the association between nutrients and micronutrients with PTL. Vitamins, protein and micronutrients likeiron have caused in decrease in haemoglobin, severe malnutrition and weight loss in maternal and foetus health[13,30,50]. Furthermore, calcium deficiencies cases had shown postpartum and neuromuscular injuries, osteoblast and osteoclast to both mother and foetus.

Table 1
Associated risk factors of Preterm labor

Risk Factors	
Environmental	Increased Xenobiotic, Petrochemical Agents, Non-oxidative pollutants (sulphur dioxide, carbon dioxide, Chlorofluorocarbons, etc.), Methane
Sociological	Race, Age, Marital Status, Economical Status, Habits etc.
Biological	
➢ Microbial	Bacteria's (vaginosis trichomoniasis, tuberculosis) Viruses:(HIV, gonorrhoea, syphilis. Parasitic: (malaria, worm infestation)
➢ Blood	Haemophilia, eclampsia, preeclampsia, anaemia
➢ Reproductive	Uterine rupture, Vaginal Bleeding, Placenta Previa, In vitro fertilization
➢ Genetic/ Metabolic	Familial history with PTL, polymorphisms (CYP51A1, HMGCR etc.), immunological (CDK and interleukin) Homocysteine and folate (MTHFR, SHMT, TYMS etc.)
➢ Nutritional	Vitamin, protein, iron, calcium, fatty acids, minerals (decreased haemoglobin, malnutrition, weight loss, gestational diabetes mellitus)

Similarly, in some cases, high consumption of carbohydrate, fatty foods etc. has increased blood glucose level, which ends up with gestational diabetes mellitus. Other nutrients deficiencies such as phosphorus, fatty acids and minerals have also been reported as a cause of PTL because of improper dietary habits[1,17,21].

Several risk factors have been identified (Table 1) associated with PTL such as environmental, sociological and biological.

Homocysteine metabolism: Homocysteine is a non- protein and sulfur-containing amino acid. The formation of homocysteine is from a combination of methionine and cysteine which is an organic sulfur found in humans[16]. Methionine adenosyltransferase (MAT) catalyzes by the biosynthesis of S-adenosylmethionine (SAM) from methionine and ATP. MAT is encoded through genes showing a tissue-unique expression pattern. MAT1A encodes MAT I/III and is only expressed in a person's liver, whereas MAT2A-expressing MAT II is present in nearly all tissues.

The SAM group donates DNA, RNA, proteins and neurotransmitters to a methyl group, for example there may be more than 100 different methyltransferases[6,15,16]. Each of these reactions generates a potent inhibitor to S-adenosylhomocystein (SAH) which hydrolyzes SAH to adenosine and homocysteine (SAHH). As the synthesis of SAHH promotes the production of SAH, both homocysteine and adenosine must be metabolized or transferred out of the cell to stop the build-up of SAH.

The remethylation of homocysteine by the enzyme MTR[6] catalyses and connects the folate cycle with the metabolism of homocysteine. The resulting complex cobalamin Cob(I) MTR, which links to 5-methyltetrahydrofolate recognized (N_5-MTH), is methylcob(III) MTR. MTR includes co-factor of Cob. When the methyl group is converted to homocysteine, the Cob (I) MTR will be modified to another N_5-MTH. Cob(I), an inactive Cob (II)MTR complex can also be oxidized to cob(II)alamine. The Cob (II)MTR complex is revitalized by reductive methylation with SAM as a donor by methionine synthase reductase (MTRR) although MTR is omnipresent, another remethylation mechanism for homocysteine is betaine homocysteine methyltransferase (BHMT) which is expressed primarily throughout the liver and the kidneys.

The molecule of homocysteine is sustained during remethylation and transmethylation reactions, but homocysteine is irreversibly reduced to cysteine in the transsulfuration process. Two vitamin B6-dependent enzymes: cystathionine β synthase (Cβs) and cystathionine β-lyase (Cβl) are facilitated through the action of transsulphurization. The hydrolysis of cystathionine to cysteine and α-Ketobutyrate is then catalyzed from Cβs for condensing homocysteine and serine to cystathionine and then Cβl. Human Cβs is expressed in the liver, kidneys, muscles, brain and ovary and also in neural and cardiac systems during early embryogenesis.

Cysteine is also a precursor of glutathione, a reliable antioxidant and a vital compound for xenobiotic detoxification, in addition to its function in protein synthesis. Homocysteine is re-methylated or exported from the cell in most tissues through methionine synthase.

The liver is the key organ for methionine degradation and retains homocysteine at sufficient concentrations through a special enzyme sequence including MAT I / III, Cβs, Cβl, BHMT, GNMT. MAT I / III which has a high km as compared to MAT II, that results in increased SAM in high methionine in the liver. High levels of SAM inhibit MTHFR and activate Cβs activity respectively and it is a major regulatory mechanism. Excess methionine thus occurs in the transsulfuration process by higher concentrations of SAM in homocysteine degradation. In comparison, if the level of methionine is less while fasting, for example, the low SAM levels will not activate Cβs or inhibit MTHFR and re-methylate homocysteine.

Folate and Homocysteine: N_5-MTH acts intracellularly as a methyl donor for re-methylation of homocysteine. Along with serine hydroxymethyltransferase (SHMT), the resulting tetrahydrofolate (THF) can be converted directly into 5,10-methyleneTHF(N_5, N_{10}-MTHF). SHMT is an enzyme that uses serine as a single carbon donor and is dependent upon B6 vitamin. SHMT has both a cytosolic and a mitochondrial isoform in humans.

Conversion of THF into the trifunctional enzyme methylenetetrahydrofolate dehydrogenase (MTHFD1) with the action of formyltetrahydrofolate synthetase, methenyltetrahydropolase and MTHFD is catalyzed by 10-formylTHf and N_5, N_{10}-MTHF. For purine biosynthesis, the 10-formilTHF may give one-carbon groups while 5, 10-MTHF can be used for the transformation of the dUMP to dTMP.

The latter reaction is catalyzed by the enzyme of thymidylate synthase (TYMS) and produces dihydrofolates (DHF) which allows the action of dihydrofolate reductase (DHFR) to reduce to THF afterwards. As well as being a cofactor for dTMP synthesis, the riboflavin-basic methylenetetrahydrofolate reductase (MTHFR), competing for N_5, N_{10}-MTHF with TYMS, can also be reduced to 5-MTHF. To limit available N_5-MTHF for homocysteine remethylation, the function of the MTHFR enzyme is very significant[6].

Conclusion

Preterm labour is one of the complex and rapidly growing health burdens in the developing world of which the causative factors are still uncertain. As there are unclear gaps for its etiological factors and there has been always a

limitation in its preventive measures therefore, this health disorder would be a challenging one for the researchers. In addition to several maternal complication and risk factors, it can be considered as a predisposition to preterm. The study of homocysteine has introduced the association between metabolic (biochemical) and genetic factor's relationships with PTL. Since the association between homocysteine and PTL has been established decades ago, very few researchers targeting the same have been conducted until now.

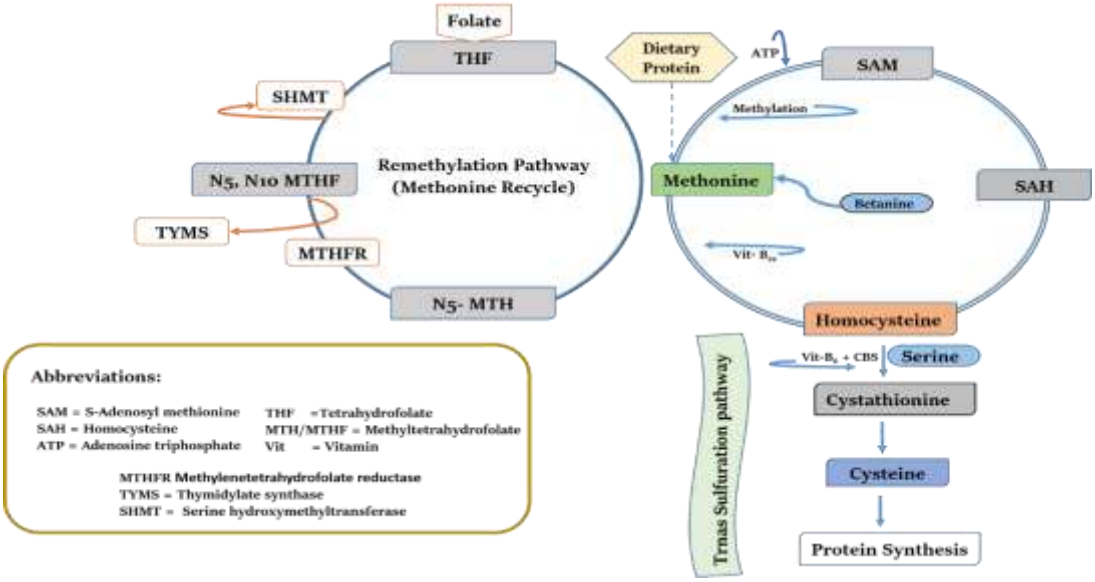

Figure 1: Homocysteine Pathway

Table 2
Decade Studies on Homocysteine and Preterm Labour

Experimental/ Investigative study groups	Methodology	Inference
A nested case-control study with 300 pregnant women	Serum levels of Homocysteine, TC, LDL-C, UA, FAA and SOD were analysed biochemically.	Elevated maternal serum homocysteine level showed to have an association with preterm birth and neonatal body weight[34]
Cohort study, with a preterm group aged 28-35 years of age (n=200) singleton mothers	Mothers with threatened preterm were selected Foetal heart rate, uterine contraction, vaginal examination and overnight plasma homocysteine level was assessed.	Maternal serum homocysteine and uterine artery pulsatility index (pi) had shown an association between preterm group[29]
Cohort study with a total number of 100 antenatal women between 8 and 12 weeks of gestation	Venous blood samples were taken after overnight fasting and processed for chemiluminescence assay. The routine examination included blood pressure recordings, pedal oedema, urine albumin and sugar, Symphysio Fundal Height and clinically amniotic fluid level status. Glucose challenge test and oral glucose tolerance test and antenatal Ultrasound was assessed	Increased homocysteine level in the first trimester of gestation resulted in hypertension, loss of the pregnancy, low birth weight, preterm birth, meconium-stained amniotic fluid[27]
in vitro study (mice)	Western blot, immunohistochemistry and overnight homocysteine level were assessed	hyperhomocysteinemia caused preterm labour in mice due to upregulation and pharmacological blockage[43]
87 pregnant women (30 term preeclampsia 27 preterm pre-eclampsia ,and 30 term controls) with singleton pregnancy were included.	Left-arm hourly blood pressure was assessed for 7 days Placental tissue was processed for immunoassay, for demethylation ELISA was done.	Increased level of homocysteine and vitamin (b12) were observed in preterm and preeclampsia women and no association has shown for maternal plasma with birth weight had resulted[22.]

Through this review, we conclude that homocysteine metabolism has an association with preterm labour. Further investigations are essential to fully understand the role of homocysteine in maternal, foetal and neonatal health and their clinical consequences.

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