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Review

Understanding The Role of Vitamin-B12 and Folic Acid in Megaloblastic Anaemia

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ABSTRACT

Vitamin B12 and folic acid are key maternal micro nutrients that work together either as enzyme or as co-enzyme for erythropoiesis, DNA methylation, histone modification, microRNAs regulation and proper functioning of iron in the body. Also, these two micro nutrients works with vitamin B6 and vitamin B9 to maintain homocysteine level in blood. They are used in combination for the treatment or prevention of variety of diseased conditions such as infertility, diabetes, cancer, liver diseases, anemia, dermatitis etc. Non- vegetarian products are rich source of vitamin B12 whereas folic is present in natural food such as green leafy vegetables, fruits, grains etc. Deficiency of both the Vitamin B12 and folic acid significantly affect DNA synthesis which is indicative of delayed nuclear maturation and an altered cell division in megaloblastic anaemia. Megaloblastic anaemia also results from microcytosis in bone marrow due to the deficiency of Vitamin B12 and folic acid. The reason behind the deficiency in every age group is poverty and lack of awareness. Several studies strongly support the reason behind megaloblastic anaemia due to impaired DNA synthesis is deficiency of the folate and vitamin B12. Though studies has been also reported that folate and vitamin B12 deficiency alter the epigenetics regulation leading to cancers. But this hypothesis is no strongly supported by any researchers. So, further more study is required to explore the relationship between megaloblastic anaemia, nutritional deficiency and epigenetic regulations.

KEYWORDS: Megaloblastic anemia; folate; vitamin B12; epigenetics; DNA methylation; MiRNAs

BACKGROUND

The bone marrow megaloblastosis are a heterogeneous group of disorders due to impaired DNA synthesis having abnormalities in morphology and features in the haematopoietic bone marrow cells. Impaired DNA synthesis results in defect nuclear maturation which affects cell division in case of megaloblastic anaemia. Haemoglobin synthesis proceeds normally, while delay in nuclear division results in production of an enlarged cell [1,2]. Folic acid play major role for the DNA synthesis and vitaminB12 help as co-factor (methyl cobalamin) in the hematopoietic red cell precursors[3]. Macrocytosis in the bone marrow can be megalobastic anemia due to deficiency of folic acid and vitaminB12 or either non -megaloblastic anaemia seen in various diseases, for example, it can be seen in chronic liver disease, dyserythropoeitic anaemia, pure red cell aplasia, aplastic anaemia and hypothyroidism. Hypersegmented neutrophils, anisocytosis and myelodysplastic syndrome are much more common in megaloblastic anaemia than non megaloblastic anaemia. The most common causes of megaloblastic anaemia in the developing countries results from nutritional deficiency such as folic acid and vitaminB12 [2-4].

On the basis of human bone marrow examination, there are mainly two types of anaemia namely, megaloblastic and non-megaloblastic anaemia. The examination of peripheral blood smear as well as bone marrow aspirates and biopsy gives morphological features of RBCs that can provide inkling to the etiology of anaemia [2,3].

Megaloblastic anaemia

It is most commonly associated with nutritional deficiencies, vitamin B12 and folate, and some other causes are like bone marrow disorders myelodysplasia, acute leukaemia, drugs and chronic illness. These micronutrients are required as cofactors for DNA synthesis and normal maturation of all cells. Bone marrow aspirate smear shows hyper cellular characteristic with all myeloid series of cells but erythroid cells being dominated in megaloblastic anaemia. The characteristic of erythroblasts are large oval shape, tear drop cells, lacy nucleus. These are the features of megaloblastic anaemia and mostly suspected to be associated with vitamin B12 or folate deficiency.

Non- megaloblastic anaemia

This type of abnormalities has been reported in the bone marrow of other hematological disorders such as blood disorder like aplastic anaemia, red cell aplasia, myelodysplastic syndromes, myeloid leukaemia ; severe hypothyroidism and some medication such as azathioprine,

Table -1: Prevalence of megaloblastic anaemia as year, age group and reference in Indian study.

YEAR	AGE GROUP	REFERENCE
1965	All ages	Bhende et al[5]
1969	Child	Mittal et al[6]
1989	All ages	Sarode et al [7]
1998 1998	Children Preschool	Gomber et al[8,9]
2001	Child	Chaudhary MW et al[10]
2002	Children	Chandra et al[11]
2005 2007	All ages All ages	Khanduri et al[12,13]
2015	All ages	Sanket K Mahajan et al[14]

Table – 2: Prevalence of megaloblastic anemia as year, age group and reference in international study.

YEAR	AGE GROUP	REFERENCE
1992	All ages	Mukibi et al[15]
1995	All ages	Maddood - ul et al[16]
1995	All ages	Allen et al[17]
1997	Lactating	Casterline et al[18]
2005	Pregnancy	Garcia – casal et al[19]

severe hyperglycaemia, hypernatremia, stored blood, pregnancy, sideroblastic anaemia, copper deficiency anaemia and cold agglutinins that are is not associated with vitamin B12 or folate deficiency but looks like similar to megaloblastic [2,3].

Prevalence as per age group in different studies

The Prevalence in India and Across the Globe

The incidence of megaloblastic anaemia is more common in developing countries due to the poor socio – economics class and appears to be increasing since over the last two decades.

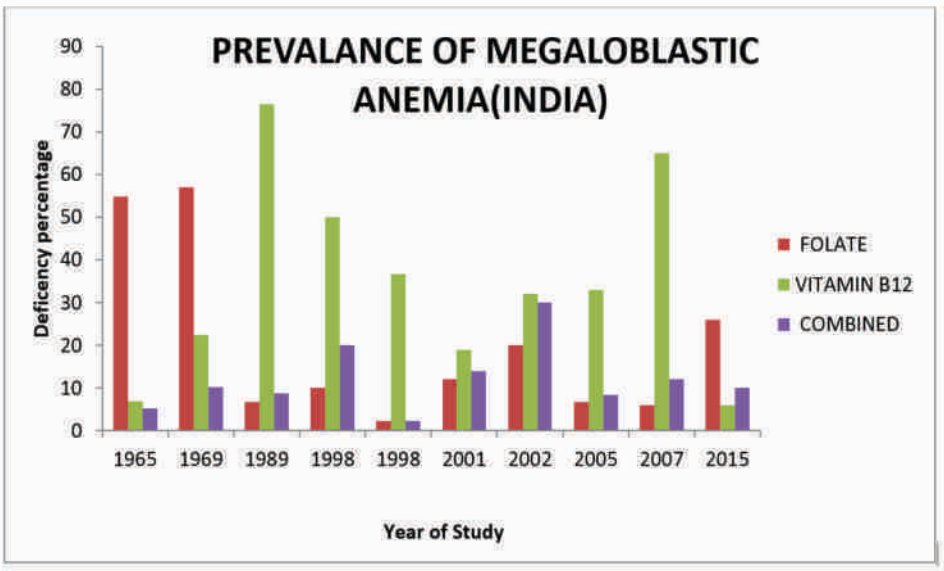

Figure 1: Prevalence of megaloblastic anemia as deficiency percentage of folate, vitaminB12 and combined in Indian study.

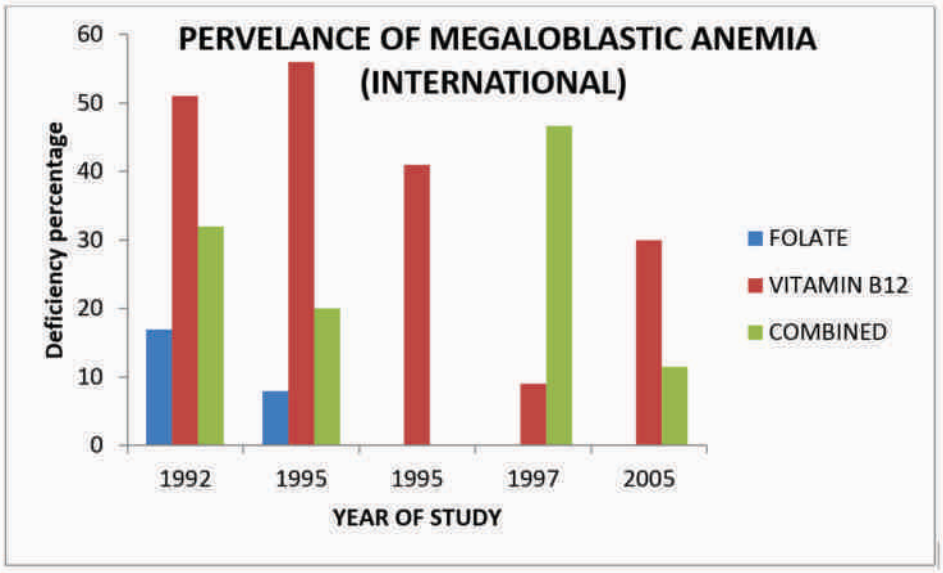

Figure 2: Prevalence of megaloblastic anemia as deficiency percentage of folate, vitaminB12 and combined in international study.

The nutritional deficiency vitamin B12 and folate play major role in the causes of megaloblastic anaemia. The study Jadish Chandra et al has reported 85% vitB12 deficiency in megaloblastic anaemia 4, whereas Thakur N et al have reported 30.5% of megaloblastic anaemia out of 131 cases [20]. Another study from Mysore, Rangaswamy et al has reported 33% of megaloblastic anaemia out of 100 cases [21].

Economic burden due to megaloblastic bone marrow disease in India and worldwide

The burden of anaemia is the major public health problem affecting 1.62 billion people globally. Estimated prevalence in high developed countries is 9% and low developed countries are 43% of anaemia. Worldwide prevalence of anaemia 25% was reported by WHO on global data base 1993 – 2005 [22]. The highest prevalence of anaemia in the world is the south Asia, due to high rates of malnutrition. According to the National Family Survey reported that prevalence of anaemia was 52% in the Indian women samples. Nutritional deficiency is one of the vital causes of megaloblastic and microcytic anaemia in developing countries. Therefore diets deficient in vitamin B12 and folate are the major causes of megaloblastic anaemia in the developing countries such as in India, due to malabsorption, infections, poverty, might result in burden of megaloblastic anaemia. Although there are several studies on anaemia but there is no any strong supporting data nationally and internationally on burden of megaloblastic anaemia[23].

Factors inducing vitamin B12 deficiency

Nutritional factor– vegans (who do not prefer animal products such as meat, fish, eggs, cheese etc in their diet); malabsorption; gastritis – functional abnormality, intrinsic factor deficiency; intestinal causes- like total gastrostomy, tropical sprue, parasitic infection - fish tape worm; the conditions which favours VitaminB12 deficiency- Zollinger – Ellison syndrome, alcohol, radiotherapy, hereditary intrinsic factor deficiency; intrinsic receptor disorder – like Imerslund grasbeck syndrome; disorder of transcobalamin 2, CbIC, CbIE and, CbIG are the factors associated with vitamin B12 deficiency [24,25].

Factors inducing Folate deficiency:

Dietary deficiency, alcoholism, infancy, old age, poverty, scurvy and kwashiorkor are major causes of folate deficiency in children and adults. Increased utilization or loss of folate can be seen in lactation, pregnancy, prematurity. Hematologic diseases (like sickle cell anaemia, thalassemia, and chronic haemolytic anaemia), malignant diseases (E.g. leukaemia, myeloma and lymphoma), inflammatory diseases (E.g. tuberculosis, crohn's disease, psoriasis, exfoliative dermatitis) and metabolic diseases (E.g. homocysteinuria) are also associated with folate deficiency. Increased urinary loss of folate has been reported in haemodialysis. Barbiturates, sulphasalazine, tetracycline, metformin, primidone, phenytoin and nitrofurantoin are the antifolate drugs that show folate deficiency [25,26].

Disease Symptoms, Association and Hematological Findings

The disease symptoms includes like shortness of breathing, fatigue, palpitations, exacerbation of angina, vein thrombosis, homocysteinuria, lymphoblastic leukaemia, acute myeloid leukaemia, weight loss, diarrhoea, constipation, anorexia, glossitis, angular cheilosis, jaundice(unconjugated) neural tube defects [24,26].

Neurological dysfunction

Peripheral neuropathy, paraesthesia, muscle weakness, dementia, psychotic disturbances, visual impairment, psychiatric disturbances.

Haematological findings

Oval macrocytes, anisocytosis, poikilocytosis, MCV >100 FL (femtoliter), neutrophils are hyper segmented.

Bone marrow findings

Hyper – cellular, giant as well as abnormal metamyelocytes.

Chromosomes

Irregular splits, decreased in concentration developing of centromere, over prominent satellites, interfere with DNA replication.

Ineffective Hemopoiesis

Urine urobilinogen, reduced haptoglobins, positive urine hemosiderin, raised LDH (lactate dehydrogenase).

Methods of managing megaloblastic anaemia

Megaloblastic bone marrow anaemia or cobalamin and folate deficiencies could be treated by understanding and identifying symptoms correctly so as to select the significant dose, duration and route of vitamin supplement. Course – megaloblastic bone marrow anaemia is very quick reversible disease which could be reverses to normal within 2- 3 days if treated and given vitamin supplements. Haematopoiesis, lactate dehydrogenase, bilirubin, hypersegmentation of neutrophil, platelet, reticulocytosis, RBC count and MCV all will be almost normal within 2 weeks of treatment [25].

Treatment Response

After treatment homocysteine & methylmalonic acid levels decrease within a few days which indicate the monitor response. Improvement in vitamin levels and reticulocyte count can also be used to monitor response. Blood transfusion can be done in severe anaemia to compensate 25.

Vitamin Supplements

If cobalamin deficiency 1000 micro – gram injections is usually given during first weeks for the reversal of cobalamin deficiency. Daily oral doses of 1000 to 2000 micro-gram should be given. Folate deficiency - Folate is given as daily doses of 1 mg, its cheap stable and very well absorbed. Large doses are requiring for children with inborn errors of metabolism.

Patient Education

Patient awareness regarding causes, course, doses, and disease associated with megaloblastic anaemia and prognosis is necessary to educate the patients [26].

Role of vitamin-B12 and folic acid in the production and maturation of erythrocytes

The Process of erythrocyte production and maturation

The haematopoietic stem cells give rise to LTRC(long term repopulating cell), CRU(competitive repopulating unit), CFU- mix(GEMM) granulocyte – erythroid – macrophage – megakaryocytic colony forming unit, spleen colony forming unit (CFU-S), erythroid burst forming unit (BFU-E), erythroid colony forming unit (CFU-E) these stem cells lead to maturation of different committed progenitor cells resulting in mature blood cell erythrocyte and the whole process of red cell production and maturation in the bone marrow is called erythropoiesis. Early phase of erythropoiesis is erythropoietin independent where as late phase of erythropoiesis is dependent on erythropoietin. Erythropoietin (EPO) is a glycoprotein hormone which is the main hormonal regulator for the production of red cell [25].

Colony forming unit – Erythroid (CFU – E) morphologically emerges as immature cells having fine nuclear chromatin, a well-defined nucleolus, a high nuclear cytoplasmic ratio and basophilic cytoplasm. A phase of active DNA synthesis of CFU-E takes place in S phase. CFU-E is fully dependent on erythropoietin and its most erythropoietin sensitive cell. Compare to CFU-E, Burst forming Unit – Erythroid (BFU-E) is much more immature and it's almost similar to multipotent hematopoietic stem cell. BFU-E morphologically appears as very immature oval shaped blast with moderately basophilic cytoplasm, very fine nuclear chromatin and immense nucleoli. It has been well established that proliferation and differentiation of BFU-E at the early stages are erythropoietin independent.

Stages of normoblastic proliferation and differentiation

Ehrlich was the first person to define term erythroblasts in to two series normal (normoblastic series) and pathologic (megaloblastic series). A cell takes around 12 to15 days at the BFU- E stage to mature in to erythroblasts. Around 6 to 8 days required for proliferation and differentiation of in BFU-E to CFU-C. Another 5 to 7 days required for proliferation and appears as basophilic erythroblasts. During this period CFU-E undergo three to five successive divisions. Probably 3 to 5 cell divisions also take place during the maturation of erythroid precursors. 8 to 32 mature red cells are formed from each pronormoplast and the cell division stop at polychromatophilic stage.

Normal series of erythroblasts maturation Proerythroblast

Proerythroblast is large in size (14 to 19 μm diameter), round to oval in shape with substantial nucleus that covers about 80% of the cell, and a rim of basophilic cytoplasm. It also contain and very small amount of haemoglobin.

Basophilic erythroblast

It is almost similar to proerythroblast but differs in size (12 to 17 μm diameter), nucleoli are not visible, in this stage condensation of chromatin and formation of heterochromatin begins. Cytoplasm appears deeply basophilic and maximum number of ribosomes appears.

Polychromatophilic erythroblast

During this stage size becomes smaller (12 to 15 μm diameter), nucleoli are not visible and more condensed nuclear chromatin. As haemoglobin increases, mitochondria decrease in this stage.

Orthochromatic erythroblast

It is the smallest (8 to 12 μm diameter) in size, nucleus undergoes pyknotic degeneration, shrinks and chromatin becomes greatly condensed.

Reticulocyte

It's larger compare to mature erythrocytes 20% greater in volume. Reticulocyte contains ribosomes, mitochondria and the Golgi complex. Staining with methyl alcohol and supravital stain as the ribosomal RNA appear blue or grey in colour. As it contains reticulum therefore, it is termed as reticulocyte.

Erythrocytes

The human mature erythrocyte is a membranous structure. Due to the lack of nucleus, mitochondria and ribosomes in erythrocytes, red cells are unable to synthesis protein. The major cytoplasmic protein is haemoglobin almost 95%. The shape of mature red cell is like biconcave disc and its size is 7 to 8 μm in diameter with central pallor. The normal subjects mean cellular volume range from 85 to 91 fl.

Factors that influence erythrocyte maturation

Erythron within normal limits is maintained by well-balanced mechanism which mediates the response to normal & abnormal conditions. Therefore any changes in the blood haemoglobin concentration, alters tissue oxygen tension that causes hypoxia in kidney and in response the kidney secretes erythropoietin hormone to maintain the balance in the red cell production. Erythropoiesis is stimulated by tissue hypoxia. Therefor tissue oxygen receptors located within the kidney induced the production of erythropoietin hormone (EPO) to regulate the erythropoiesis.

Erythropoietin

It is a glycoprotein hormone produced by the kidney and liver which regulated the red cell production. The molecular weight is 34,000 Daltons. It is located on human chromosome 7. It has four introns, five exons and 193 amino acid polypeptide. Erythrothropoietin binds to the erythropoietin receptors that are located on cell surfaces. And the expressions of both are necessary for adult life. In immature erythrocytes an intracellular signal is generated by the activation of the EPOR that protects these cells from apoptosis. As the BFU-E matures to CFU-E, EPORs increases and highest EPORs can be seen at the stage between CFU-E to proerythroblast. At the stage of

orthochromatic erythroblast, the EPORs disappear and are unable to express on reticulocytes or red blood cells. DNA cleavage proceeds rapidly to apoptosis in absence of erythropoietin while the presence of EPO prevents cell death and it allow the erythroid cells to undergo differentiation process so as to form mature red cells.

Key roles played by vitamin B12 and folic acid in the maturation of erythrocytes

The normal maturation of cells (erythrocytes) require adequate amount of folate and vitamin B12. The folate is necessary for the denovo synthesis of pyrimidines (thymine, cytosine, uracil) and purines (adenine & guanine) these nucleotides required to assemble the DNA. At the stage of mitosis folate is very necessary for DNA synthesis, if deficiency of folate or inhibition of folate metabolism shows or arrests the proliferation of erythropoiesis. The lack of folate and vitamin B12 inhibit the thymidylate synthetase which leads to decrease in deoxythymidine triphosphate synthesis, resulting in formation of excess 2- deoxyuridine triphosphate. Formation of excess 2- deoxyuridine triphosphate leads incorporation of 2- deoxyuridine triphosphate in to DNA and ultimately results in DNA strand breaks and cell death (apoptosis)[27].

Mechanisms regulated by vitamin-B12 and folic Acid DNA Methylation

DNA methylation in the genome is a covalent modification in which there is an addition of methyl group (CH3) in cytosine within 5′- CpG - 3′ dinucleotide and S. adenosylmethionine (SAM) helps to transfer methyl group to form 5- methyl cytosine. Methylation of DNA never alters the DNA coding sequence. The methylation occurs at the CpG rich promoter regions [28]. Study has reported (Krista S. Crider et. al) that 4.25% of total cytosine in genomic DNA are methylated in in primary human fibroblast cell line, 67.7% of CpGs are methylated and 99.98% of DNA methylation occurs in CpG dinucleotide, 25% of all cytosine methylation takes place at non – CpG sites. The methylation of CpG islands in the promoter region is a normal process that silences gene expression to regulate gene expression in cells. The gene silencing at the CpG island connect specific methylated DNA binding proteins for example DNA methyltransferases, transcription factors, DNA methyl binding proteins , histone modifying enzymes , chromatin remodelling factors. These all proteins/ enzymes and DNA interactions bring changes in DNA conformation to form chromatin that inhibits messenger RNA and protein formation [29].

Folate, Vitamin B12 and DNA methylation

Variety of dietary factors which are associated with one carbon metabolism act as co-enzymes by influencing the supply of methyl groups for the methylation process of DNA, RNA, neurotransmitters, phospholipids, proteins and histones30. When these nutrients reach to intestine and liver 5-methyltetrahydrofolate is formed, 5-methyltetrahydrofolate can be used directly by non-hepatic tissues. This 5-methyltetrahydrofolate will convert to tetrahydrofolate (THF) through the methionine synthase reaction. With help of the help of the enzyme dihydrofolate reductase, Folic acid reduced to dihydrofolate and then to THF so as to enter the folate pool [31]. Thus formed THF this converted to 5, 10 methylene THF by the enzyme serine hydroxymethyltransferase. The enzyme methylene tetrahydrofolate reductase reduces 5, 10 methylene irreversibly to 5- methyl THF. The vitamin B12 dependent methionine synthase reaction maintains the flux of methyl groups for the remethylation of homocysteine to methionine. S- Adenosylmethionine (SAM) formed from methionine transfer its methyl groups for all possible methylation reactions, including the methylation of DNA, RNA and proteins.

Apart from folate there are some other dietary nutrients such as vitamin B6, riboflavin, vitamin B12 and choline that are required to maintain one carbon metabolism, normal homocysteine remethylation, SAM formation and DNA methylation. This is the how one carbon metabolism works. MTHFR is inhibited when there is high concentration of SAM. This result in reduced synthesis of 5- methyl THF, and influences remethylation of homocysteine. Genetically altered MTHFR activity and 5- methyl THF formation reduces enzyme activity. S-Adenosyl homocysteine (SAH) acts as a product inhibitor for SAM – dependent methyltransferase. So the hydrolysis of SAH to homocysteine is necessary to regulate normal DNA methylation process. Several studies have been reported that low folate dietary intake or diseases associated alter the folate absorption for multiple cancers, neural tube defects and neurological symptoms. During the DNA replication and cell division folate has major role to play. Inadequate availability of folate during cell division can result in the compromised production of thymidine. This is the reason that uracil are more incorporated in the DNA sequence which may cause defect in DNA repair and rises the frequency of chromosomal breaks. Study has been reported that low folic acid concentration in tissue culture results in formation of micronuclei and MTHFR TT genotype influence the formation of micronuclei under low folate conditions. Chronic myeloid leukaemia (CML) is associated with two genes MTHFR polymorphisms, C677T and A1298C, are involved with reduced enzyme activity. MTHFR is an important enzyme which catalyses the reduction of 5, 10 methylenetetrahydrofolate to 5-methylenetetrahydrofolate in folate and homocysteine metabolism and generates methyl groups for DNA methylation. MTHRF polymorphisms (C677T, 1298CC) have shown risk of developing chronic myeloid leukemia (CML)[32]. Folate plays important role for the synthesis, repair and methylation of DNA. Folate deficiency causes DNA strand breaks, reduced DNA repair and aberrant DNA methylation. Also, the increased risk of childhood leukemia[33] is associated with low folate level. Although several studies have been reported the role of folate and vitamin B12 in DNA methylation in different types of cancers such as colorectal, liver, lungs, skin, cervical, breast and haematological malignancy but there is no enough data for role of folate and vitamin B12 for DNA methylation in

megaloblastic anaemia. Some study has reported no DNA methylation in megaloblastic anaemia[34] but study has been reported that folic acid deficiency impair the formation of S-adenosylmethionine which can affect the methylation process causing megaloblastic anaemia as well as several other pathologies[35].

Epigenetic regulation

Epigenetics is the modifications in the gene expression. It occurs in the initial stage of carcinogenesis. There is a major role of dietary compounds on mechanism influencing the epigenome[36]. The micronutrients which play major role in the modifications of epigenetics include folate, vitamin B12, retinoic acid and selenium. Normal cellular functions and development is control by the epigenetic mechanism.

DNA methylation

DNA methylation is the process where methyl groups transferred from S- adenosylmethionine (SAM) to the 5′ - position of cytosine - with the help of enzyme DNA methyltransferases[28,29]. DNA methylation during DNA replication is maintained by methyltransferase such as DNMTs (DNMT1, 3a and 3b), DNMT1 play major role in DNA methylation. It methylate mainly newly synthesized un-methylated DNA strand to assure transmission of DNA methylation patterns to daughter cells.

Histone modification

Post – translational modifications such as phosphorylation, ubiquitinylation, ADP ribosylation, acetylation, methylation and cell cycle checkpoint integrity that occurs at the N-terminal tails of histones are associated with regulation of gene expression. Modifications in sequence – specific transcription factors or histone modifying enzymes are most investigated. Histone acetylation and histone methylation have been reported with neoplastic formation. The two enzymes - histone acetyltransferase and histone deacetlases play major role to maintain acetylation of histones tails that opens the chromatin structure and allows transcription factors to access the DNA consequently proteins [36]. Chromatin condensation and transcriptional repression occurs due to histone deacetylation. At lysine and arginine residues histone methylation takes place. Histone lysine methylation play role in deregulation and up regulation of gene expression. The methylated lysine residue such as H3K4, H3K36 and H3K79 has been associated with transcriptional active chromatin (euchromatin) but H3K9, H3K27 and H4K20 are associated with transcriptional inactive heterochromatin. In the methylation process of histone lysine, histone lysine methyltransferases donate its methyl group from S-adenosylmethionine to the lysine-residue[36,37].

Vitamin B12 and folic acid regulates microRNAs

Folate and vitamin B12 deficiency leads to genetic variation and alter the normal one carbon metabolism. One carbon dysfunction has been associated with several diseases such as cancer, megaloblastic anaemia and neural tube defects [38]. MicroRNAs (miRNAs) have -22 nucleotide RNAs which play key regulatory roles in gene families in eukaryotes. MicroRNAs are non - coding RNA molecule (22 nucleotides) present in eukaryotes which regulate RNA silencing and post – transcription in gene expression. 24521 microRNAs have been discovered to date, in that human genome encodes over 2000 different miRNAs [39,40]. Studies have been reported that folate influences microRNA expression through one carbon metabolism. One carbon metabolism genes MTHFR, TCbIR, TCN2, SLC19A1, MAT2A and MTHFD2 are regulated by microRNAs -22, which are involved in folate and vitamin B12 transport as co- factors and methylation. Apart from microRNAs – 22, some other miRNAs which regulate one carbon metabolism genes include microRNAs 125/351, microRNAs 344-5P/484 and microRNAs 488. Changes in the expression of microRNAs such as microRNA 29c, microRNA 34a, microRNA 155 and microRNA 200b were reported in mice with methyl deficient diet in liver cancer. MicroRNA 222 over expression was reported in folate deficient human in vivo study. It has also been reported that folate deficiency in human lymphoblastic cell line induces changes in the expression of microRNAs such as hsa- microRNA 22, hsa – microRNA 145, hsa- microRNA 146, hsa –microRNA 345 and hsa –microRNA 205 and folate supplementation can suppress ethanol induced toxicity [41]. Even though there is several studies reported that folate and vitamin B12 alter the microRNA gene regulation in different cancer. Most of the studies have shown that folate deficiency can alter one carbon metabolism by microRNA regulation associated with different one carbon metabolism genes which is indirectly supporting the megaloblastic anaemia but there is no any direct supporting data for alteration in microRNAs in megaloblastic anaemia.

Co-Enzyme functions of vitamin B12

Cobalamin or vitamin B12 also called as cyanocobalamin, having tetrapyrrole ring with cobalt atoms and nucleotide side chains is together called corrin. Vitamin B12 in the form of Adenosyl cobalamin and methylcobalamin functions as co-enzyme in human system. L methylmalonyl – CoA mutase enzyme required for L- methylmalonyl CoA to convert it in to succinyl CoA. Another reaction methionine synthase required as enzyme for homocysteine to convert it in to methionine. The result methionine is utilized in the protein synthesis, methyl donor, S- adenosylmethionine (SAM), which act as supplier of methyl groups during the methylation process of DNA, RNA, and Proteins etc [42].

Co- enzyme functions of folic acid

Folic acid is composed of pteroic acid having pteridine ring attached to a P- aminobenzoic acid residue. Folate is required in the one carbon metabolism and synthesis of purines, deoxythymidine etc. It play major role as co-enzyme in the one-carbon metabolism. Folate in the liver with the action of dihydrofolate reductase (DHFR) converted to dihydrofolate, subsequently with action of NADPH enzyme converted to tetrahydrofolate (THF). Tetrahydrofolate during oxidation or reduction process form different one – carbon units such as

methylene –THF (help in synthesis of thymidine), formyl –THF (utilized in the synthesis of DNA, RNA and proteins), methyl – THF (help in the formation of methionine utilized in the formation of S-adenosylmethionine called methyl donors used for methylation of DNA, RNA and proteins etc.

Folate and vitamin-B12 deficiency

In one carbon metabolism as a co-enzyme folate participates in the nucleotide synthesis, DNA methylation and help to prevent impaired DNA synthesis which will lead to megaloblastic anaemia 43. Folate deficiency limits the intracellular levels of tetrahydrofolate co-enzyme. Cobalamin deficiency fixes the intracellular supply of tetrahydrofolate co-enzyme by trapping folate in the 5- methyl tetrahydrofolate. There is methylation of deoxyuridylate to thymidylate with the help of co-enzyme 5, 10 methylenetetrahydrofolate (methylene THF) that transfers methylene group. But in megaloblastic anaemia there is decreased conversion of deoxyuridylate to thymidylate in cells, resulting in increased uracil misincorporation in to DNA, the reason decreased purine and thymidylate synthesis which will increase apoptosis and alter cell cycle [44].

Clinical evidences demonstrating supplementation of vitamin B12 or folic acid for treating megaloblastic bone marrow diseases

Megaloblastic anaemia most commonly defined as deficiency of vitamin B12 and folate. This has been established treatment with appropriate vitamins supplement. Once the severely ill patient is diagnosed by blood samples or bone marrow biopsy (if required) for cobalamin and folate deficiency, the large dose of both B12 and folate are given to patient. If the patient is severely affected then packed red cells should be given [26].

Cobalamin deficiency treatment

The patient with cobalamin deficiency for life long should be given cobalamin injections regularly. The signs and symptoms of megaloblastic anaemia that results from the deficiency of B12 and folate are well documented. The deficiency of cobalamin due to fish tape worm and intestinal stagnant loop can be treated permanently. The persons who are having borderline deficiency of cobalamin should be followed up and given the supplement. For the patient with gastrectomy or ileal resection, it is ideal to inject recommended dose of hydroxocobalamin injections IM as cobalamin supplement to complete the body replenishment[26].

Folate deficiency treatment

The folate is well absorbed even from patient having malabsorption. Satisfactory doses of folic acid range from 5 – 15 mg daily and therapy should be given for 4 months. If cobalamin deficiency is there first it should be corrected and then folate supplement should be given otherwise cobalamin neuropathy may develop. The patients should be given advice to have good sources of folate containing diets after correction of folate deficiency. It is recommended to check serum folate and cobalamin levels at regular intervals to know the status of deficiency. Reduced form of folate called folinic acid given usually to minimize the harmful effects of dihydrofolate reductase inhibitors. Food fortified with folic acid called as prophylactic acid has been given in many countries to prevent neural tube defects. It also reduces homocysteine levels to prevent cardiovascular diseases. Around 400 µg of folic acid supplement is recommended daily to pregnant women to prevent neural tube defect in fetus and after pregnancy 5mg is recommended. Infancy and childhood recommended 1mg daily supplement.

CONCLUSION

Several studies have been reported and well established that folate and vitamin B12 deficiency causes megaloblastic anaemia due to impaired DNA synthesis. Some studies has been reported that folate and vitamin B12 deficiency alter the epigenetics regulation (DNA methylation, Histone modification and microRNAs regulation) leading to cancers. But there is no direct evidence or strong supporting data to support this hypothesis. So, further more study is required to illuminate the relationship between megaloblastic anaemia, nutritional deficiency and epigenetic regulations.

REFERENCES

1.Veda P. Evaluation of macrocytosis in routine hemograms. IJHBT 2013 ; 29(1):26-30.

2.Aslinia F, Mazza JJ, Yale SH. Megaloblastic anemia and other causes of macrocytosis. J Clin Med Res 2006 Sep 1; 4(3):236-241.

3.HAQ SS, Iqbal N, Fayyaz F, Tasneem T. Serum B12 and folate levels in patients with megaloblastic change in the bone marrow. Biomedica 2012; 28(1):35-39.

4.Chandra J. Megaloblastic anemia: back in focus. Indian J Pediatr 2010 ;77(7):795-799.

5.Bhende YM. Some experiences with nutritional megaloblastic anemia. J Postgrad Med 1965 ; 11(4):145-155.

6.Mittal VS, Agarwal KN. Observations on nutritional megaloblastic anemias in early childhood. Indian J Med Res 1969; 57:730-738.

7.Sarode R, Garewal G, Marwaha N, Marwaha RK, Varma S, Ghosh K, Mohanty D, Das KC. Pancytopenia in nutritional megaloblastic anaemia. A study from north-west India. Trop Geogr med 1989 ; 41(4):331-336.

8.Gomber S, Kela K, Dhingra N. Clinico-hematological profile of megaloblastic anemia. Indian J Pediatr 1998 ; 35:55-58.

9.Gomber S, Kumar S, Rusia U, Gupta P. Prevalence & etiology of nutritional anaemias in early childhood in an urban slum. Indian J Med Res 1998 Jun 1; 107:269.

10.Chaudhary MW. Clinico-hematological study of nutritional anemia in young children (Doctoral dissertation, Thesis for MD Pediatrics, Delhi University).

11.Chandra J, Jain V, Narayan S, Sharma S, Singh V, Kapoor AK, Batra S. Folate and cobalamin deficiency in megaloblastic anemia in children. Indian J Pediatr 2002 ; 39(5):453-457.

12.Khanduri U, Sharma A, Joshi A. Occult cobalamin and folate deficiency in Indians. Natl Med J India 2005 ; 18(4):182.

13.Khanduri U, Sharma A. Megaloblastic anaemia: prevalence and causative factors. Natl Med J India 2007;20(4):172-175.

14.Mahajan SK, Aundhakar SC. A study of the prevalence of serum vitamin B12 and folic acid deficiency in Western Maharashtra. Fam. Med. Prim. Care Rev 2015 Jan; 4(1):64.

15.Mukibi JM, Makumbi FA, Gwanzura C. Megaloblastic anemia in Zimbabwe: spectrum of clinical and haematological manifestations. East Afr Med J 1992; 9: 83-87

16.Mannan M, Anwar M, Saleem M, Wiqar A, Ahmad M. A Study of Serum Vitamin B~ 1~ 2 and Folate Levels in Patients of Megaloblastic Anaemia in Northern Pakistan. J Pak Med Assoc 1995 ; 45:187.

17.Allen LH, Rosado JL, Casterline JE, Martinez H, Lopez P, Munoz E, Black AK. Vitamin B-12 deficiency and malabsorption are highly prevalent in rural Mexican communities. Am J Clin Nutr 1995 Nov 1; 62(5):1013-1019.

18.Casterline JE, Allen LH, Ruel MT. Vitamin B-12 deficiency is very prevalent in lactating Guatemalan women and their infants at three months postpartum. J Nutr 1997 ;127(10):1966-1972.

19.Garcia-Casal MN, Osorio C, Landaeta M, Leets I, Matus P, Fazzino F, Marcos E. High prevalence of folic acid and vitamin B12 deficiencies in infants, children, adolescents and pregnant women in Venezuela. Eur J Clin Nutr ; 59(9):1064-1070.

20.Thakur N, Chandra J, Pemde H, Singh V. Anemia in severe acute malnutrition. Nutrition. NLM 2014 ; 30(4):440-442.

21.Rangaswamy M, Nandini NM, Manjunath GV. Bone marrow examination in pancytopenia. J. Indian Medical Assoc 2012 ; 110(8):560-562.

22.Balarajan Y, Ramakrishnan U, Özaltin E, Shankar AH, Subramanian SV. Anaemia in low-income and middle-income countries. The lancet 2011 ; 378(9809):2123-2135.

23.Bentley ME, Griffiths PL. The burden of anemia among women in India. Eur J Clin Nutr 2003 ; 57(1):52-60.

24.Kaferle J, Strzoda CE. Evaluation of macrocytosis. AAFP 2009 ; 79(3):203-208.

25.Greer JP, Arber DA, Glader B, List AF, Means RT, Paraskevas F, Rodgers GM. Wintrobe's clinical hematology. Lippincott Williams & Wilkins; 2013 Aug 29.

26.Fauci AS, Braunwald E, Kasper DL, Hauser SL, Longo DL, Jameson JL, Loscalzo J. Harrison's manual of medicine. Tata McGraw Hill Education publisher 2009.

27.Koury MJ, Ponka P. New insights into erythropoiesis: the roles of folate, vitamin B12, and iron. Annu Rev Nutr 2004 ; 24:105-131.

28.Crider KS, Yang TP, Berry RJ, Bailey LB. Folate and DNA methylation: a review of molecular mechanisms and the evidence for folate's role. Adv Nutr 2012 ; 3(1):21-38.

29.Davis CD, Uthus EO. DNA methylation, cancer susceptibility, and nutrient interactions. Exp Biol Med 2004 ; 229(10):988-995.

30.Stover PJ. One-carbon metabolism–genome interactions in folate-associated pathologies. J Nutr 2009 ; 139(12):2402-2405.

31.Bailey RL, Mills JL, Yetley EA, Gahche JJ, Pfeiffer CM, Dwyer JT, Dodd KW, Sempos CT, Betz JM, Picciano MF. Unmetabolized serum folic acid and its relation to folic acid intake from diet and supplements in a nationally representative sample of adults aged ⩾ 60 y in the United States. Am. J Clin Nutr 2010 ; 92(2):383-389.

32.Aly RM, Taalab MM, Ghazy HF. MTHFR A1298C and C677T gene polymorphisms and susceptibility to chronic myeloid leukemia in Egypt. Int J Clin Exp Pathol 2014; 7(5):2571.

33.Metayer C, Scélo G, Chokkalingam AP, Barcellos LF, Aldrich MC, Chang JS, Guha N, Urayama KY, Hansen HM, Block G, Kiley V. Genetic variants in the folate pathway and risk of childhood acute lymphoblastic leukemia. Cancer Causes & Control 2011 ; 22(9):1243-1258.

34.Perry J, Chanarin I, Deacon R, Lumb M. Methylation of DNA in megaloblastic anaemia. J Clin Pathol 1990 ; 43(3):211-212.

35.Ifergan I, Assaraf YG. Molecular mechanisms of adaptation to folate deficiency. Vitamins & Hormones 2008 ; 79:99-143.

36.Ali AA, Shalhoub SS, Cyr AJ, Fitzpatrick CK, Maletsky LP, Rullkoetter PJ, Shelburne KB. Validation of predicted patellofemoral mechanics in a finite element model of the healthy and cruciate-deficient knee. J. Biomech 2016 ; 49(2):302-309.

37.Choi SW, Friso S. Epigenetics: a new bridge between nutrition and health. Adv Nutr 2010 ; 1(1):8-16.

38.Stone N, Pangilinan F, Molloy AM, Shane B, Scott JM, Ueland PM, Mills JL, Kirke PN, Sethupathy P, Brody LC. Bioinformatic and genetic association analysis of microRNA target sites in one-carbon metabolism genes. PloS one 2011 ; 6(7):e21851.

39.Valinezhad Orang A, Safaralizadeh R, Kazemzadeh-Bavili M. Mechanisms of miRNA-mediated gene regulation from

common downregulation to mRNA-specific upregulation. Int J Genomics 2014 Oct; 2014.

40. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004 ; 116(2):281-297.

41. Wang LL, Zhang Z, Li Q, Yang R, Pei X, Xu Y, Wang J, Zhou SF, Li Y. Ethanol exposure induces differential microRNA and target gene expression and teratogenic effects which can be suppressed by folic acid supplementation. Human Reproduction. 2009 ; 24(3):562-579.

42. Burtis CA, Ashwood ER, Bruns DE. Clinical Chemistry and Molecular Diagnostics. Tietz fourth edition. St. Louis, Mo.: Elsevier Saunders publisher 2006.

43. Kim KC, Friso S, Choi SW. DNA methylation, an epigenetic mechanism connecting folate to healthy embryonic development and aging. J Nutr Biochem 2009 ; 20(12):917-926.

44. Koury MJ, Price JO, Hicks GG. Apoptosis in megaloblastic anemia occurs during DNA synthesis by a p53-independent, nucleoside-reversible mechanism. Am J Hematol 2000 ; 96(9):3249-3255.