**T.R.N.C**

**NEAR EAST UNIVERSITY**

**GRADUATE SCHOOL OF HEALTH SCIENCES**

# ROLE OF VITAMIN $B_{12}$ AND FOLATE DURING PREGNANCY

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**MEDICAL BIOCHEMISTRY PROGRAM**

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**SUPERVISOR**

**Associate Professor Özlem DALMIZRAK**

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**2017**

The Directorate of Graduate School of Health Sciences,

This study has been accepted by the project committee in Medical Biochemistry Program as a Master Project.

Project committee:

| | |
|---|---|
| Chair: | Professor Nazmi Özer<br>Near East University |
| Supervisor: | Associate Professor Özlem Dalmızrak<br>Near East University |
| Member: | Assistant Professor Eda Becer<br>Near East University |

Approval:

According to the relevant articles of the Near East University Postgraduate Study – Education and Examination Regulations, this project has been approved by the above mentioned members of the project committee and the decision of the Board of Graduate School of Health Sciences.

Professor İhsan Çalış
Director of the Graduate School of Health Sciences

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# ABSTRACT

**Zaydi ZM. Role of Vitamin $B_{12}$ and Folate During Pregnancy. Near East University, Graduate School of Health Sciences, Graduation Project in Medical Biochemistry Program, Nicosia, 2017.**

Vitamins act to facilitate many of the body's mechanisms and functions. Thus, deficiency of vitamins causes many health problems. This study summarizes the role of folate and vitamin $B_{12}$ in pregnancy. Folic acid has a great importance for pregnant women and nursing mothers, so folate deficiency causes complications for mothers. In newborn often folate levels are balanced because they rely on mother's stores, however folic acid deficiency in the mother may cause some complications and problems that may affect the health status of births. Vitamin $B_{12}$ also plays an important role in the health of mothers and fetuses. Vitamin $B_{12}$ deficiency during pregnancy causes many problems such as early miscarriage and birth defects.

**Key words:** Folate, vitamin $B_{12}$, pregnancy, birth defects

# ÖZET

**Zaydi ZM. Gebelikte Vitamin $B_{12}$ ve Folatın Rolü. Yakın Doğu Üniversitesi, Sağlık Bilimleri Enstitüsü, Tıbbi Biyokimya Programı, Mezuniyet Projesi, Lefkoşa, 2017.**

Vitaminlerin vücuttaki mekanizmaları ve işlevleri kolaylaştırıcı görevi bulunmaktadır. Bu nedenle vitamin yetersizliği çeşitli sağlık sorunlarına yol açmaktadır. Bu proje folat ve $B_{12}$ vitamininin gebelikteki rolünü özetlemektedir. Folik asit gebe ve emziren kadınlar için oldukça önemlidir ve folat eksikliği annelerde komplikasyonlara neden olmaktadır. Yeni doğanlar beslenme bakımından anneye bağlı olduğundan folat düzeyi dengelenmektedir. Ancak annedeki folik asit eksikliği yeni doğanın sağlık durumunu etkileyebilecek komplikasyonlara ve problemlere neden olmaktadır. $B_{12}$ vitamini anne ve fetus sağlığında da önemli rol oynamaktadır. Gebelikte $B_{12}$ eksikliği düşük ve doğum kusurları gibi birçok probleme neden olmaktadır.

**Anahtar Kelimeler:** Folat, $B_{12}$ vitamini, gebelik, doğum kusurları

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| | |
|---|---|
| ABG | : Atrophic body gastritis |
| BHMT | : Betaine-homocysteine methyltransferase |
| CBS | : Cystathionine B–synthase |
| CI | : Confidence interval |
| DFE | : Dietary folate equivalents |
| DHF | : Dihydrofolate |
| DHFR | : Dihydrofolate reductase |
| dTMP | : Deoxythymidine monophosphate |
| dUMP | : Deoxyuridine monophosphate |
| EAR | : Estimated average requirement |
| HCY | : Homocysteine |
| HELLP | : Hemolysis elevated liver enzyme low platelets |
| IF | : Intrinsic factor |
| MMA | : Methylmalonic acid |
| MTHFD1 | : Methylenetetrahydrofolate dehydrogenase 1 |
| MTHFR | : Methylenetetrahydrofolate reductase |
| MTR | : Methionine synthase |
| MTRR | : Methionine synthase reductase |
| NTD | Neural tube defects |
| OR | : Odds ratio |
| PABA | : p-aminobenzoic acid |
| RBC | : Red blood cell |
| RDA | : Recommended dietary allowance |
| RFC1 | : Reduced folate carrier 1 |
| SAH | : S-adenosylhomocysteine |
| SAM | : S-adenosylmethionine |
| SHMT | : Serine hydroxylmethyltransferase |
| TCI | : Transcobalamin 1 |

| | |
|---|---|
| TCII | : Transcobalamin 2 |
| TCIII | : Transcobalamin 3 |
| THF | : Tetrahydrofolate |
| WBC | : White blood cell |

# LIST OF FIGURES

## 1. INTRODUCTION

The significance of folic acid in reproductive age can be valued through considering that the lack of enough vitamin is the main factor of megaloblastic anemia in pregnant women. Nowadays, low level of maternal folic acid in child-bearing period and lactation remains a basic explanation behind maternal morbidity in a small group. Low folic acid levels during pregnancy might prompt to lower weight of newborn. Clinical vitamin $B_{12}$ shortage might be a reason behind impotence or intermittent regular abortion. In early stages of pregnancy, having an insufficient vitamin $B_{12}$ situation might be a risk of childbirth defects, for instance neural tube defects (NTD), and it can also lead to premature birth. Likewise, lacking vitamin $B_{12}$ status in the pregnant woman makes neonate a candidate of vitamin $B_{12}$ deficient even if the satisfactory fetal storage of vitamin $B_{12}$ is not set down in early pregnancy period or not sufficient in mother's milk. While folate insufficiency has been recognized for decades as the most widely recognized reason for macrocytosis in conception, the vitamin is known to have noteworthy and broad role in reproduction (Tamura and Picciano, 2006).

Folic acid prevents neural tube defects (NTD) and there are procedures to minimize these defects by administering folic acid nutrition (Anne et al., 2008). Its function in declining the frequency of congenital deformities is now analyzed. The impetus generated by the evidence of NTD counteractive action has urged researchers to reconsider a large group of other antagonistic pregnancy events, many of which were suspected for a very long while to be connected to maternal folate status. On the opposite side, the impacts of minimum vitamin $B_{12}$ status in reproduction are not all around characterized, in spite of the fact that there are relative metabolic connections amongst folate and vitamin $B_{12}$. This might be somewhat because of confirmation that serious vitamin $B_{12}$ insufficiency, as shown in pernicious anemia, can bring about infertility, additionally, vitamin $B_{12}$ insufficiency is usually thought to be an insufficiency of the elderly and subsequently the outcomes of insufficient or borderline vitamin $B_{12}$ situation on child growth occasionally has spoken. Notwithstanding, these perspectives are being adjusted. Eating habbit (e.g., vegetable) sometimes customary leads to minimum vitamin $B_{12}$ levels in some part of world and are turning out to be

more common in different ranges. It is getting to be perceived that there is extremely higher worldwide predominance of low vitamin $B_{12}$ level between women and children than heretofore assumed (Allen, 2005). Therefore, reports on the impacts of nutritious vitamin $B_{12}$ inadequacy on maternal and neonatal wellbeing are seeming all the more frequently in the literature. In this project we search the evidence relating insufficient vitamin $B_{12}$ and folate levels together with pregnancy.

## 2. GENERAL INFORMATION

### 2.1. Overview of Vitamins

Vitamins are vital nutrients for different processes in human body, assuming a key role in numerous chemical processes for the normal working of the body. In decades, it has been illustrated that vitamins are significantly important in wellbeing and human ailments (Mamede et al., 2011). In this study we introduce the importance of cobalamin and folate during pregnancy.

### 2.2. Vitamin B Complex

The vitamin B complex contains many water soluble vitamins and includes vitamin $B_1$ (thiamin), $B_2$ (riboflavin), $B_3$ (niacin), $B_5$ (pantothenic acid), $B_6$ (pyridoxine), $B_7$ (biotin), $B_9$ (folic acid) and $B_{12}$ (cobalamin). They all exist in eggs, nuts, brewer's yeast, milk, fish, rice, meat, liver, organic products, verdant green vegetables and numerous different nourishments. These vitamins preserve and enhance the metabolism rate, protect muscle tone, ensure the great state of the dermal tissues, enhance nervous and immune systems and advance development and cell division. The function of B vitamins in the protection and therapy of cancer is uncertain, as the outcomes are in rare evidence and conflicting publications exist in literature (Mamede et al., 2011).

Folic acid and cobalamin both function in methylation reactions in human body. Methionine synthase, a vitamin $B_{12}$-dependent enzyme, stimulates the exchange of a methyl group from methyltetrahydrofolate to homocysteine to give methionine, in this manner guaranteeing the arrangement of S-adenosylmethionine (SAM), the essential methyl group donor for most physiological methylation reactions, inclusive that of DNA (Kim, 2005). Deficiency of vitamin $B_{12}$ and folic acid decreases the accessibility of SAM and inhibits DNA methylation. Therefore, gene expression and conformation of DNA as well as normal control of the expression of protooncogenes are influenced (Rogers, 1995; Scott, 1999).

**2.3. Folate**

Folic acid, a non-specific term for the water soluble $B_9$ vitamins, participates in single-carbon transfer reactions and is found in numerous chemical forms. The most oxidized and stabilized type of folate is pteroylmonoglutamic acid which does not occur naturally in food but is utilized as a part of vitamin supplements. Folate comprises of PABA molecule connected toward one side to a pteridine ring and at the other side to one glutamic acid molecule as shown in Figure 2.1. Most abundant folic acid, pteroylpolyglutamate, contains 1 to 6 extra glutamate molecules joined in a peptide linkage to the γ-carboxyl of glutamate. Folic acid is soluble in water while excess amounts are excreted by urine, thus body does not build up folic acid stores necessitating a regular supply from daily diet. Folate acts as a coenzyme in single-carbon transfers in the metabolism of amino acids and nucleic acids. Folate bound with erythrocyte along with plasma homocysteine and folate concentration are together the main indicator for estimating the Recommended Dietary Allowance (RDA) of folate. Recommended Dietary Allowance (RDA) is 400 μg/day of dietary folic acid equivalents (DFEs) for both women and men. Dietary folate equivalents alter for about 50 percent lower bioavailability of food folic acid contrasted and that of folate: 1 μg of (DFEs) = 0.6 μg of folate from enriched nourishment or as a supplement brought with meals = 1 μg of nourishment folic acid = 0.5 μg of a supplement given on empty abdomen. To decrease the risk of neural tube defects (NTD) for women for getting pregnant, the suggestion is having 400 μg of folate every day from enriched supplements and nourishment or both of them. The evidence accessible on the part of folic acid in decreasing the risk of carcinoma, mental disturbance and vascular infection is not adequately convincing to utilize danger diminishment of these circumstances as a reason for setting the estimated average requirement (EAR) and the RDA (Pitkin et al., 1998).

The pteridine ring in the most naturally occruing folate (polyglutamates) is reduced to give either the 7,8-dihydrofolate (DHF) or 5,6,7,8-tetrahydrofolate (THF) (Bender, 2003; Bailey, 2004). In cells, reduced form of folate conjugates to the polyglutamate chain.

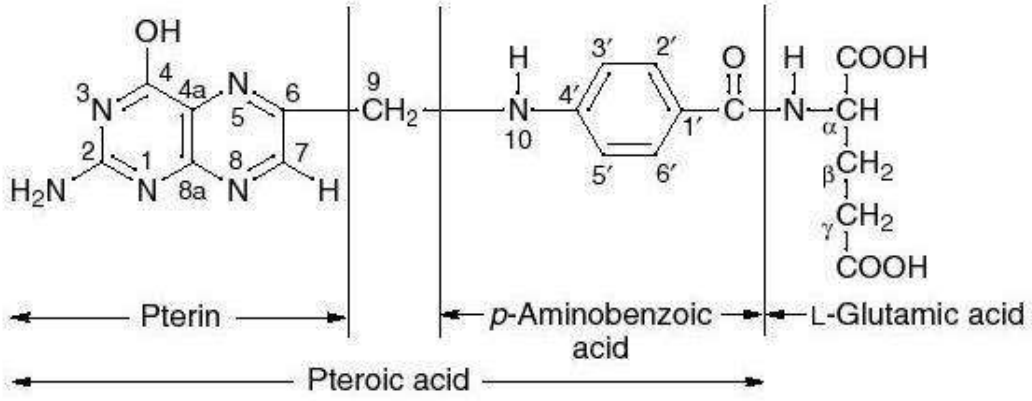


Figure 2.1. Structure of a folic acid compound. It comprises a bicyclic pterin ring joined by a methylene bridge to *p*-aminobenzoic acid which in turn attached via α-peptide to a single molecule of L-glutamic acid (Ball, 2004).

Polyglutamates have a single type of action in that they can accept so called one-carbon (1-C) units from various donors and pass them in various biosynthetic reactions. Thus, in cells folate will be a mixture of tetrahydrofolate (e.g. 10-formyl-, 5,10-methylene- and 5-methyltetrahydrofolate) depending on which C group is attached to them (Scott, 1999).

### 2.3.1. Folate Metabolism

Folate cannot be synthesized in the body, thus it must be obtained from exogenous sources. There are two sources of folate: dietary folate and folate synthesized by bacteria in the large intestine. The latter folate is directly absorbed in the colon (Ball, 2004). Before the intake of dietary folic acid, the polyglutamate chain should be broken in the mucosal cells by the enzyme conjugase (pteroylpolyglutamate hydrolase) and produced monoglutamate is taken by specific transporter located on the cell membrane of the small intestine. Much of the dietary folate undergoes methylation inside of the intestinal mucosa, therefore 5-methyl-tetrahydrofolate (which is a monoglutamate) is the only form that enters the circulation as shown in Figure 2.2 (Scott, 1999). Almost two-

third of the folic acids are protein-bound in plasma. During the first pass, the liver takes about 10–20% of 5-methyltetrahydrofolate and the rest is cleared quickly by peripheral tissues. The big part of 5-methyltetrahydrofolate reaching to the liver from the intestine is taken up unchanged. It enters the enterohepatic cycle where it is metabolized to derivatives of polyglutamate or secreted in the bile to be reabsorbed in the small intestine with food folic acid before re-entering the circulation. The kidney plays a role in maintaining body's folic acid by reabsorbing folic acid from the glomerular filtrate (Steinberg, 1984).

Before it is used or stored in tissues as coenzyme, dietary folate (monoglutamate derivates) is changed to the polyglutamate form by polyglutamate synthetase enzyme. When it gets back to the circulation, it must be changed to the monoglutamate shape. Folic acid must be converted to the polyglutamate form to function in one-carbon atom transfer in metabolism of nucleic acids and amino acids. Both cobalamin and folic acid are metabolically interrelated, that is why deficiency of one causes the same haematological changes (Pitkin et al., 1998). Folic acid secreted in the bile is reabsorbed in the small intestine, much of the folic acid in circulation is related to plasma proteins and thus cannot be filtered by kidneys. The free circulating folic acid which is filtered is captured by the folic acid receptors in the renal tubules and released into the bloodstream. The removal of folic acid by faecal or urinary excretion is very little (Bender, 2003).

### 2.3.2. Folate Function

Folate functions as a coenzyme which involves in (1) purine synthesis (forming of glycinamide ribonucleotide and 5-amino-4-imidazole carboxamide ribonucleotide); (2) DNA production, that relies on a folic acid coenzyme for pyrimidine nucleotide synthesis (methylation of deoxyuridylic acid to thymidylic acid) which is necessary for natural cell cycle; (3) generation of formate into the formate pool (and use of formate); (4) amino acid interchanges including the degradation of histidine to glutamic acid, interchanging of serine and glycine and transformation of homocysteine to methionine. Folate-mediated transfer of a carbon unit from serine gives a main source of substrate in

one-carbon metabolism. The interchange of homocysteine to methionine works as a main source of methionine for the generation of SAM, a critical methylating agent (Wagner, 1996).

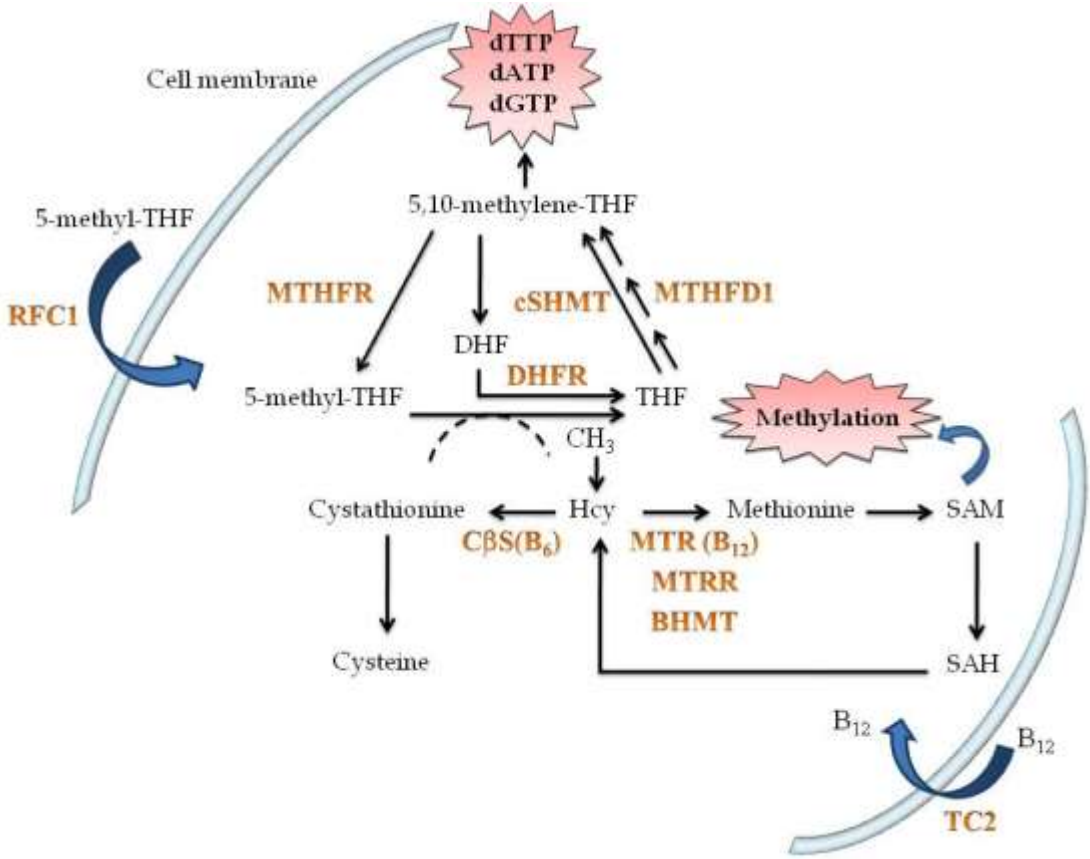


Figure 2.2. Folate metabolism. BHMT=Betaine-homocysteine methyltransferase; CβS=Cystathionine β-synthase; DHF=Dihydrofolate; DHFR=Dihydrofolate reductase; Hcy=Homocysteine; MTHFD1=Methylenetetrahydrofolate dehydrogenase 1; MTHFR=Methylenetetrahydrofolate reductase; MTR=Methionine synthase; MTRR=Methionine synthase reductase; RFC1=Reduced folate carrier 1; SAH=S-adenosylhomocysteine; SAM=S-adenosylmethionine; cSHMT=Serine hydroxymethyl transferase; TC2=Transcobalamin 2; THF = Tetrahydrofolate (Pavarino et al., 2011)

### 2.3.3. Folate Deficiency

Folic acid (vitamin $B_9$) functions with cobalamin and vitamin C to assist the body to break down, utilize and create new proteins. Folate helps to form red blood cells (RBC) and white blood cells (WBC). It likewise helps to produce DNA, the building block of the human body, which carries hereditary data. Folate insufficiency impacts embryonic development and create nonviable progeny (Ortbauer et al., 2016).

Blood disorders occurs when the levels of the RBCs or WBCs are low. Megaloblastic anemia (abnormally large RBC) is seen often in folate-deficiency anemia. Pregnant women need proper sources of enough folic acid. The vitamin is crucial in the growth of the fetus's spinal cord and formation of brain. Folic acid insufficiency may result in severe birth flaws as shown in Figure 2.3. The Recommended Dietary Allowance (RDA) for folate during conception is 600 mg/day. Nutrition Board and The Institute of Medicine Food suggest that adults should get 400 micrograms of folate every day. Women who may get pregnant ought to take folic acid supplements to guarantee that they obtain sufficient vitamin every day. Particular proposals rely on upon a man's age, sex and different elements (for example, pregnancy and lactation). Numerous edibles, for example, fortified breakfast cereals, now have additional folic acid added to forestall birth deformities (Antony et al., 2013; Antony, 2016). A balanced diet is the best way to assure receiving enough vitamins.

### 2.3.4. Folic Acid Situation in Pregnancy

Through the period of conception, there is a persistent drop in mother’s serum folic acid level to around half of non-pregnant levels (Chanarin, 1969). This is to some degree physiological reaction to conception, related to hormonal level modification, changes in renal capacity and hemodilution (Chanarin, 1969). In same manner, the standard plasma cutout levels of such vitamins might not be evident in diagnosing genuine inadequacy as by now. Extended placental and fetal requests are moreover at risk to add to this decline and also there is a confirmation of accelerated mother catabolism of folic acid as conception advances, relating with times of cell development and identical to a turnover of around 400 μg consistently before the third trimester. This

doubled folate necessity could realize a broadcasted negative folic acid equilibrium in women whose are definitely not on extra folic acid consumption throughout the conception (McNulty et al., 2013).

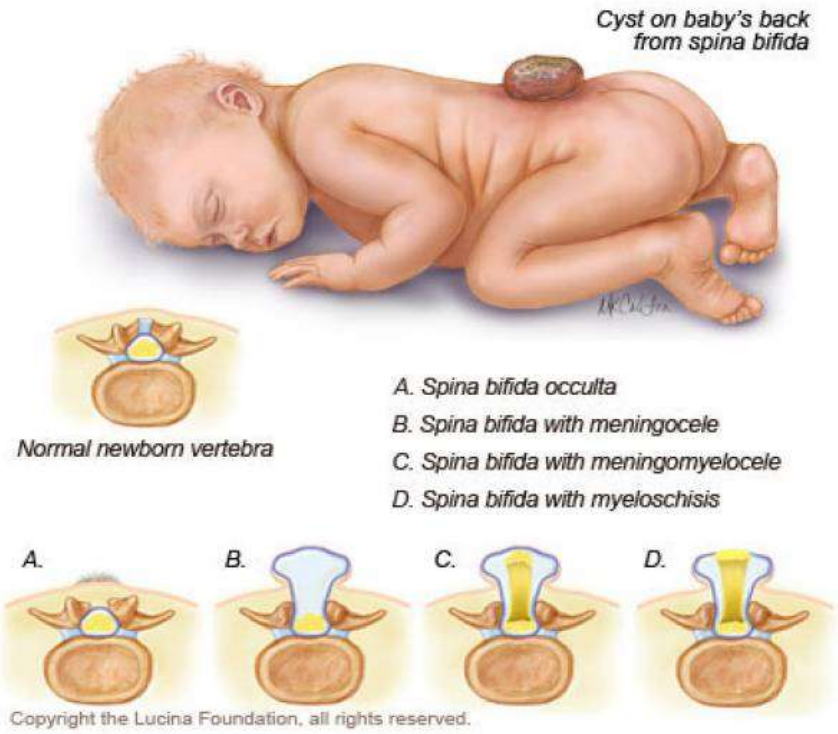


Figure 2.3. Folic acid deficiency

Elevated maternal folic acid removal from plasma and increased urinary folate discharge have likewise been accounted for yet are unrealistic to reduce stores of mothers considerably (Allen, 2005). Low interpregnancy interims may likewise add to consumption of maternal folate situation, prompting unfavorable results in later pregnancies (Megahed and Taher, 2004).

Before the far reaching utilization of pre-birth folic acid supplements at advanced nations, it was there various evidence of folic acid inadequacy and megaloblastic anemia during conception (Gatenby and Lillie, 1960). Currently, folate inadequacy relating with conception, both with and without megaloblastosis, still a general medical problem around the universe. Case reports have likewise archived serious folic acid inadequacy introducing as HELLP disorder which is characterized by breakdown of blood cells,

hoisted liver catalyst and low platelet pancytopenia (Gatenby and Lillie, 1960; Varma, et al., 2004).

Hibbard, through the 1960s expanded the study relating low folic acid and mother impacts. He proposed that folic acid insufficiency was so normal in gestation, its impacts can be observed on time of conception and were not only shown by megaloblastic anemia, as well as by early separation of the placenta, premature birth, and conceivably by fetal anemia (Hibbard, 1975). Hibbard additionally proposed that maternal low levels brought on a general weakness of fetal development which was reflected in lower weights of newborn (Hibbard, 1975). This consequence is now related with the chronic diseases (Barker, 2003). In the previous twenty years, the function of folic acid in conception has been broadened to associate a basic part in gestation period, especially at the term of neural tube closure (MRC Vitamin Study Research Group, 1991; Czeizel and Dudas, 1992). This information has provoked an analysis of the key role of folic acid in conception together in connection to avoidance of unfavorable foetal results, for example, other birth deformities and growth hindrance, and to a reassessment of the impacts of deficient folic acid situation on deteriorating conditions, for example, preeclampsia, abruptio placentae, unnatural birth cycle and unconstrained premature birth (Anne et al., 2008).

### 2.3.5. Low Folic Acid and Negative Pregnancy Results

Lacking maternal folic acid has been connected with preeclampsia, placental abruption, unconstrained embryo evacuation, low birth weight, preterm transport and stillbirth. Maybe the most grounded affiliations could be seen with early separation of the placenta. Some researchers, in the 1960s, found maximum rates of placental abruption in young women suffering from megaloblastic anemia (Hibbard, 1975). The plasma completes homocysteine fixation which is a delicate sign of folic acid levels has additionally been ensnared as a danger component for abruptio placentae and expanded danger of abruption the placenta is related to polymorphisms in folic acid related genes (van der Molen et al., 2000).

Different researches have tended to the problem of folate insufficiency in connection to stillbirth, miscarrying spontaneous and repetitive conception loss (Martin et al., 1965; Chanarin et al., 1968). Fifty percent reported the relationship between vitamin insufficiency and threat of fetal loss.

Lack of folic acid in blood was independent risk factor for spontaneous preterm birth (odds ratio [OR] = 1.47; 95% confidence interval [CI], 1.01 to 2.14) when there is an adjustment of the risk factors and other confounding variables, but high folate status was associated with no significant decrease in risk (OR = 0.74; 95% CI, 0.47 to 1.10) (Wilcox, 2001). The role played by folate in these forms is little. Early child birth, less child growth, less amount of body weight (< 2,500 g) and low birth weight (< 1,500 g) usually are major issues influencing newborn child morbidity and mortality around the world (Wilcox, 2001). So it influences premature child birth and low birth weight is related to the growth of a child. Albeit various researches have evaluated the effect on these results of mothers folate intake status or polymorphisms in folate-related genes. (George et al., 2002; Siega-Riz, 2004). The evidence for an affiliation stays delicate and questionable. Two late incomprehensible studies are important. In one examination with more than 2,000 pregnant ladies, low second trimester serum or red cell folate was found to be connected with a practically multiplied risk of preterm birth (Siega-Riz, 2004). The former research had six hundred and eighty three mothers and six hundred and fourteen neonates, red cell folate in mother during early child birth is linked to the born baby weight (Relton et al., 2005). Not having folate in child birth may lead to undesirable events. However, there was no impact on newborn weight, spontaneous abortion or gestational age of live-born infants after periconceptional multivitamin supplementation in the Hungarian randomized trial of 5,502 pregnant women (Wilcox, 2001).

### 2.3.6. Folic Acid Situation in New Born and Children

Folic acid is absorbed by fetus compartment over a center slope and the plasma concentration in newborn at conveyance is about twofold of the mother's concentration (Bruinse and van den Berg, 1985; Molloy et al., 2002). There is a solid relationship among the status in the two compartments (maternal and fetus), yet a shockingly weak

relation among fetus plasma total homocysteine and as for fetus folate or maternal levels (Molloy, 2002). This might be because of some degree to folate supplementation in conception. The blood folate concentration of breast feeding children is kept up at a significantly larger amount than in mother and insufficiency once in a while happens (Tamura et al., 1980). Regardless, there is a favorable association amongst blood folate of mothers and children amid lactation and milk folate is affected by mother's folate utilization (Ek, 1983; Salmenpera et al., 1986). In any case, even in folic acid-insufficient mothers, folic acid discharge in milk is kept up to the detriment of maternal stores (Metz et al., 1968). Lactating mothers might need an extra 300 μg folic acid daily to keep up sufficient blood folate levels (Willoughby and Jewell, 1968) and are at enormous danger of insufficiency if folic acid intake is low.

The general diet situation of youngsters is an essential fundamental issue in inclination to ailment and contamination and folate-related iron deficiency is basic in kids with HIV disease (Vilaseca et al., 2001; Bjorke and Ueland, 2003). It might likewise be a confusing component in youngsters with anemia. It is hard to determine either mother's or baby's folate inadequacy prompts reduced neural or mental function, rather such issues are trademark of errors in folate catabolism and anabolism. Regardless, there is no evident verification of such a relationship to date (Picker and Coyle, 2005). Strangely, French et al. noticed a decrease in the prevalence of neuroblastoma in youngsters after including of folate fortification in regime of children in USA (French, et al., 2003). Number of studies have related the folate related gene polymorphism in maternal as a result of the genetic disorders, like Down syndrome (Botto et al., 2004). Previously, in an investigation of 4,451 children in early puberty, mother's dietary folate consumption amid conception was fundamentally connected with mineral substance in the bones of the spine in nine years old (Tobias et al., 2005). This may propose that maternal conception folate levels may impact later formative occasions of the posterity, however it might likewise be denoting a poor maternal eating routine that is shared by the developing child.

## 2.4. Vitamin $B_{12}$

Cobalamin is one of the eight B vitamins. It has many functions for the body including the synthesis of DNA and RNA. It works with folate in the synthesis of RBC and it has role in methylation. Vitamin $B_{12}$ can be converted to one of two cobalamin coenzymes that are active in human metabolism, namely methylcobalamin and 5-deoxyadenosylcobalamin (Bender, 2003). The common synthetic form of vitamin $B_{12}$ is cyanocobalamin. It is the most stable form of the vitamin and is commonly used in pharmaceutical preparation and food supplementation (Scott, 1997; Ball, 2004). In the structure of vitamin $B_{12}$, four coordination sites on the central cobalt atom are occupied by the nitrogen atoms of the corrin ring and one nitrogen of the dimethylbenzimidazole nucleotide. Coordination sites may be occupied by N-cyanocobalamin, hydroxocobalamin, methylcobalamin and 5-deoxyadenosylcobalamin (Figure 2.4) (Bender, 2003).

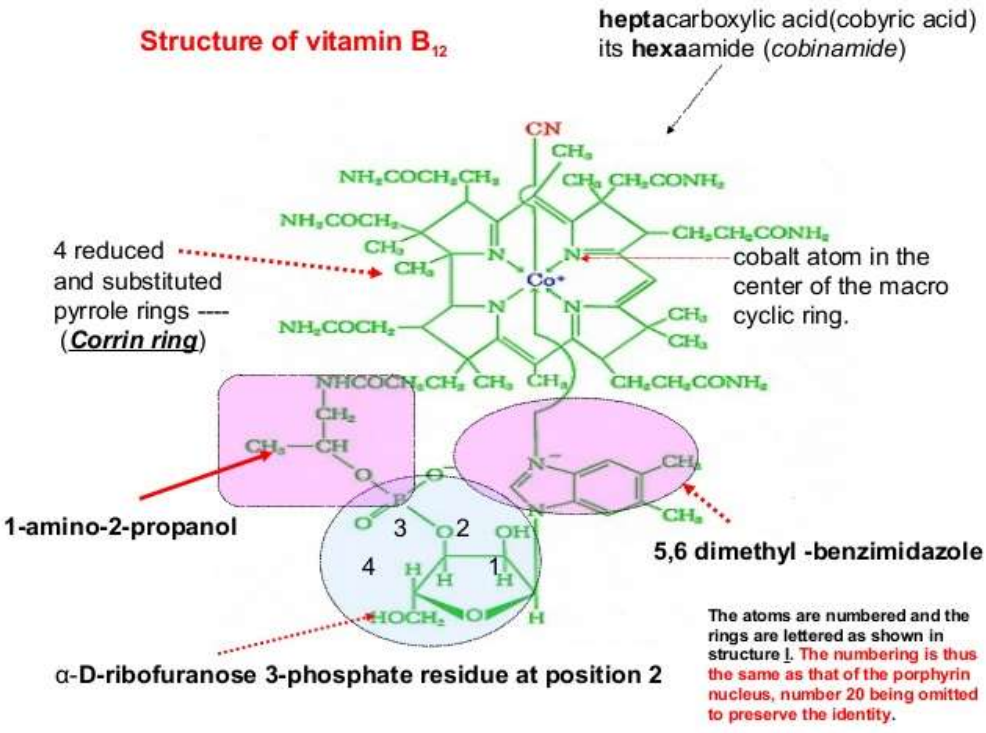


Figure 2.4. Vitamin $B_{12}$ structure (http://www.slideshare.net/banuman35/vitamin-23032364)

### 2.4.1.Metabolism of Vitamin $B_{12}$

Metabolism of cobalamin is complex and it needs many processes as shown in Figure 2.5. Any problem in these processes leads to a risk of developing deficiency (Oh and Brown, 2003). Little amount of cobalamin can be absorbed by passive diffusion through the intestinal mucosa (Andres et al., 2004). When cobalamin is absorbed, it binds to carrier proteins known as transcobalamin (TCI, TCII or TCIII) and then it is transported to all parts of the body (Andres et al., 2004). TCI prevents circulating vitamin $B_{12}$ from being filtered by the kidneys (Ball, 2004). TCII is the form which delivers cobalamin to the body tissues through specific receptors. About 50% of cobalamin is taken by the liver and the rest amount is transported to other tissues, thus vitamin $B_{12}$ is released into cytoplasm in the form of hydroxocobalamin. Hydroxocobalamin is converted to adenosylcobalamin in the mitochondria or to methylcobalamin in cytoplasm (Figure 2.6) (Andres et al., 2004). TCIII is quickly cleared in the liver, it may return cobalamin to the liver. Thus other corrinids are secreted in the bile in the form cbl-r protein complex.

Vitamin $B_{12}$ is bound to the protein in food and hydrochloric acid in the stomach releases $B_{12}$ from protein during digestion. Once released, $B_{12}$ combines with a substance called intrinsic factor (Figure 2.7) (Aghajanian and Marek, 2000). Intrinsic factor, a protein that binds avidly to dietary vitamin $B_{12}$ and promotes its transport to the terminal ileum for absorption. The deficiency of intrinsic factor is a consequence of the presence of atrophic body gastritis (ABG) resulting in the destruction of the oxyntic mucosa and thus, the loss of parietal cells which normally produce chlorhydric acid as well as intrinsic factor.

Unlike other water-soluble vitamins, the body can maintain vitamin $B_{12}$ efficiently, 80% of vitamin $B_{12}$ is stored in the liver and the rest is in the blood, skin and muscle. When circulating vitamin $B_{12}$ exceeds the capacity of binding proteins, it will be excreted in the urine, this case only after injection of vitamin $B_{12}$ (Ball, 2004).

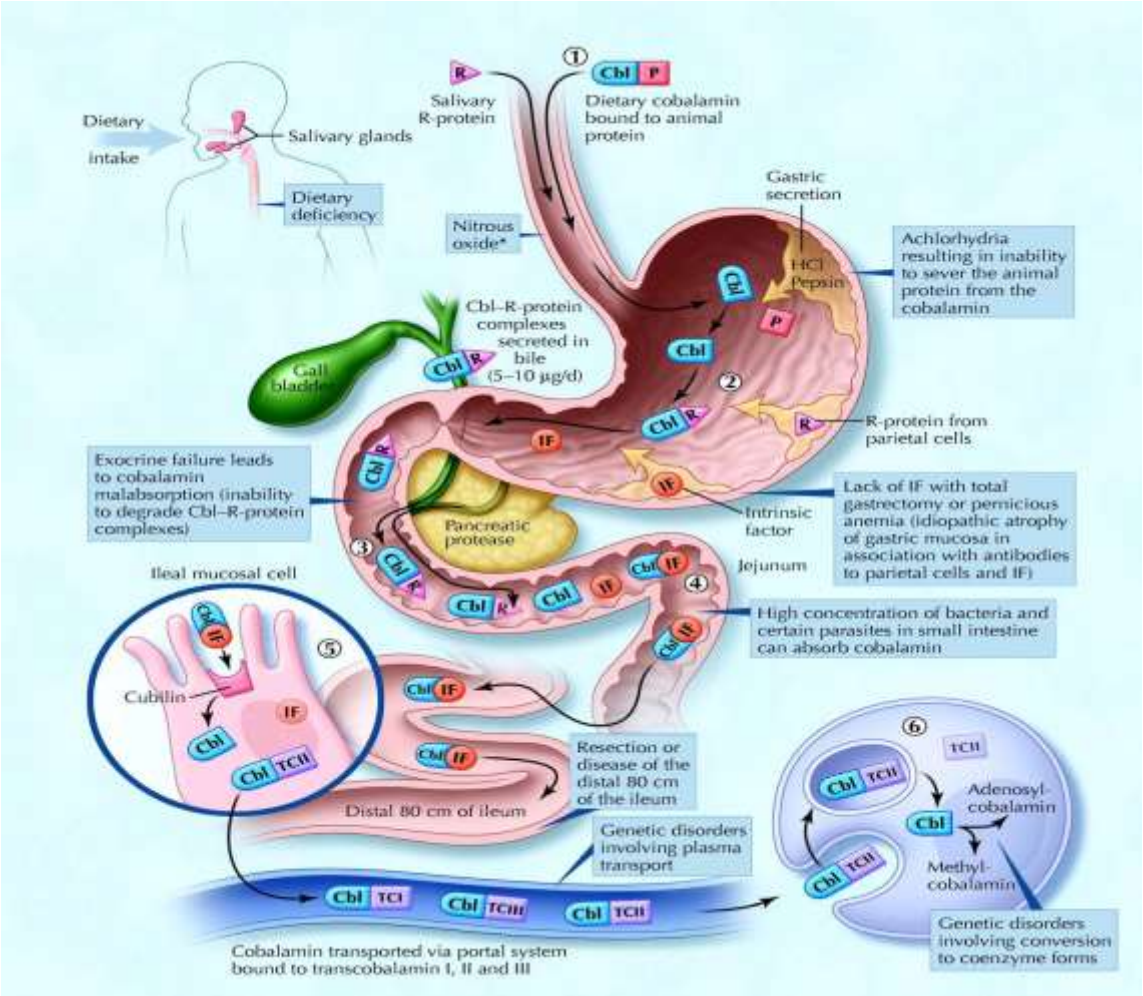


Figure 2.5. The metabolic pathway of vitamin $B_{12}$ (Andres et al., 2004)

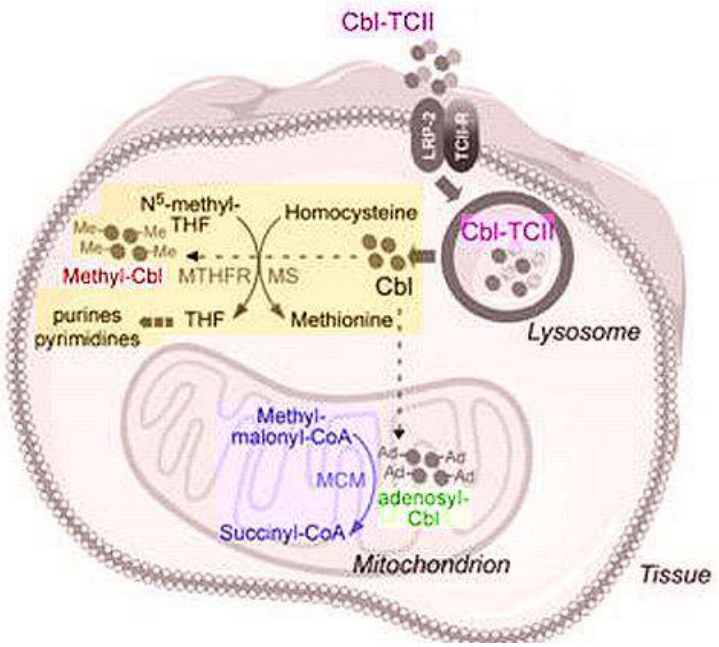


Figure 2.6. The cellular uptake and processing of vitamin $B_{12}$ (Dali-Youcef and Andrès, 2009).

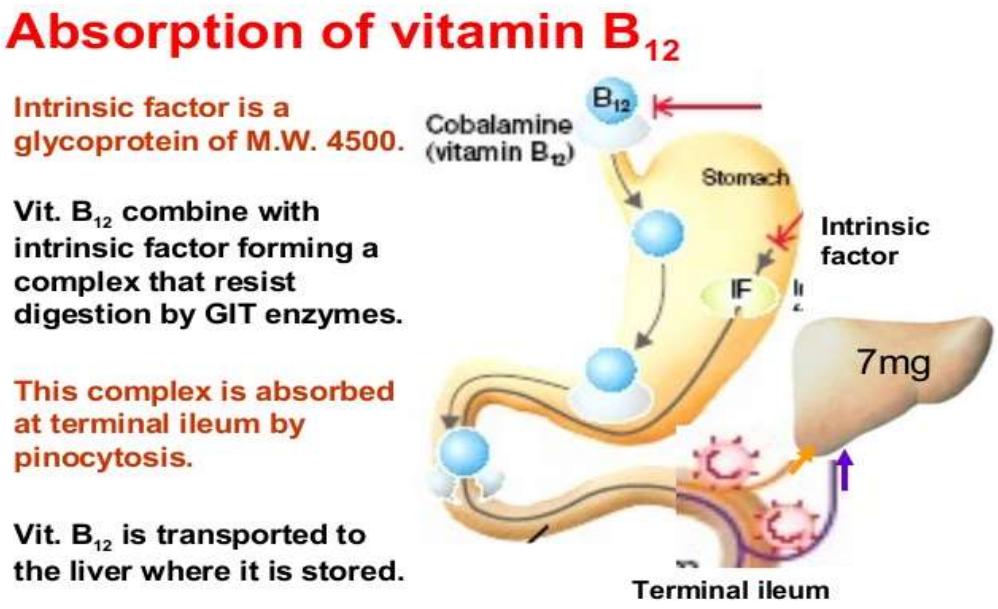


Figure 2.7. Intrinsic factor for the Vitamin $B_{12}$

### 2.4.2. Vitamin $B_{12}$ Function

Cobalamin comprises a number of forms including cyano-, methyl-, deoxyadenosyl- and hydroxy-cobalamin. The cyano form which is used in supplements, is found in trace amounts in food (Scott, 1997). The other forms of cobalamin could be changed to the methylcobalamin or 5-deoxyadenosylcobalamin forms which are required as cofactors for methionine synthase and L-methylmalonyl CoA mutase. Methionine synthase is important for the synthesis of purines and pyrimidines. The reaction depends on methylcobalamin as a cofactor and is also dependent on folate (Gibson, 2005). Methylmalonyl CoA mutase converts methylmalonyl CoA to succinyl CoA with 5-deoxyadenosyl cobalamin required as a cofactor as shown in Figure 2.8. (Gibson, 2005). The accumulation of methylmalonyl CoA is thought to be responsible for neurological effects seen in vitamin $B_{12}$ deficiency (Gibson, 2005).

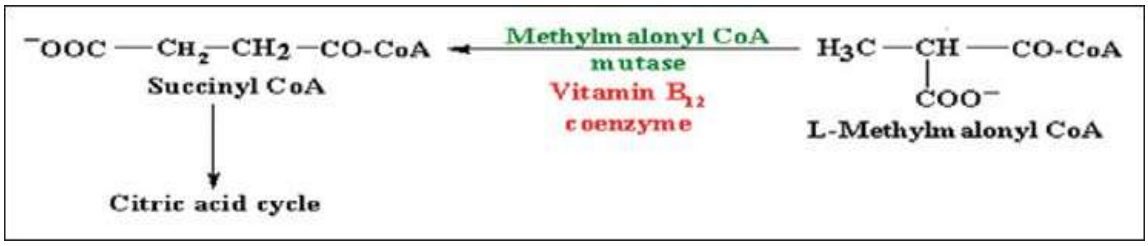


Figure 2.8. The process of obtaining succinyl CoA from L-methylmalonyl CoA in the presence of methylmalonyl CoA mutase (Glatz et al., 2010)

### 2.4.3. Vitamin $B_{12}$ Deficiency

Inadequacy of cobalamin is a typical reason for macrocytic anemia and has been concerned in a range of neurological and mental issues. The key function of $B_{12}$ insufficiency in the advancement of atherosclerosis and the hyperhomocysteinemia has been investigated. A more delicate strategy of examination for vitamin $B_{12}$ insufficiency is the estimation of serum methylmalonic acid (MMA) and homocysteine levels which are in high concentration in the beginning of vitamin $B_{12}$ insufficiency. Vitamin $B_{12}$ insufficiency is connected with hematologic, neurologic and mental indications.

Hematologic manifestations of vitamin $B_{12}$ insufficiency include pancytopenia (thrombocytopenia, leukopenia) and megaloblastic anemia. Neurologic manifestations of vitamin $B_{12}$ insufficiency can be summarized as peripheral neuropathy, combined systems disease (demyelination of dorsal columns and corticospinal tract) and paresthesias. Psychosis, dementia, mild memory impairment, depression, personality change and irritability are the mental manifestations of vitamin $B_{12}$ insufficiency (Oh and Brown, 2003).

Deficiency is caused by the malabsorption of cobalamin although dietary inadequacy is common in the elderly, vegans with poor diets. Causes can also relate to inadequate IF production, atrophic gastritis, interference with the ileal uptake of cobalamin due to disease, resection or interference by bacterial overgrowth, drug-nutrient interactions (Pitkin et al., 1998).

Pregnant and/or lactating women following vegan diets are at high risk of deficiency because of increased metabolic demand for vitamin $B_{12}$ and also gastritis or inflammation of the gastric mucosa increases with age or in some cases complete loss of the acid required to cleave vitamin $B_{12}$ from protein (Pitkin et al., 1998; National Health and Medical, 2005).

Pernicious anemia is the end stage of an auto-immune gastritis and results in the loss of IF synthesis. Loss of IF causes vitamin $B_{12}$ deficiency and if untreated, megaloblastic anemia and neurological complications develop. Pernicious anemia is treated with vitamin $B_{12}$ injections or large doses of oral vitamin $B_{12}$. Vitamin $B_{12}$ deficiency will also develop after gastric antrum resection as this is the site of secretion of IF and acid (Pitkin et al., 1998). Diseases of the ileum such as Crohn's Disease or other chronic bowel inflammatory conditions also cause malabsorption of vitamin $B_{12}$ (Pitkin et al., 1998).

### 2.4.4. Mechanism of Action of Folate and Vitamin $B_{12}$

Folic acid and vitamin $B_{12}$ are necessary for new cell formation (Kamen, 1997). Folic acid is involved in nucleic acid synthesis, methionine regeneration and in the activation, oxidation and reduction of one-carbon units, referred to as 1-C metabolism,

required for normal metabolism and regulation (Bailey and Gregory, 1999). Vitamin $B_{12}$ is an important cofactor involved in two enzymatic reactions in the cell metabolism; methionine synthase and methylmalonyl CoA mutase. Methionine synthase converts homocysteine to methionine and then to S-adenosylmethionine (SAM) in the presence of vitamin $B_{12}$ (methylcobalamin) and folate (tetrahydrofolate) (Figure 2.9). Lack of methionine synthase from vitamin $B_{12}$ insufficiency will lead to a decreased synthesis of methionine and 5-tetrahydrofolate (5THF), as well as the accumulation of homocysteine and 5-methyl-THF (5MTHF). Reduced methionine synthesis will lead to less SAM production, which is a common methyl donor required for the maintenance of methylation in DNA (Fenech and Ferguson, 2001). SAM normally prevents methylene-THF reductase (MTHFR), thus an impaired production of SAM will reduce this suppression and results in the irreversible conversion of 5,10-methylene-THF to 5-methyl-THF. The 5-methyl-THF then becomes metabolically trapped owing vitamin $B_{12}$ insufficiency. Furthermore, the reduction in 5THF leads to reduced availability of 5,10-methylene-THF, which is needed to convert deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP) in the presence of thymidylate synthase (Figure 2.9). Under conditions of folic acid insufficiency, dUMP accumulates and as a result uracil is incorporated into DNA instead of thymine (Blount et al., 1997).

Methylmalonyl CoA mutase is required for the degradation of odd-chain fatty acids and branched-chain amino acids, in particular conversion of methylmalonyl CoA in the presence of vitamin $B_{12}$ (adenosylcobalamin) to succinyl CoA, which is an important substrate in the Krebs cycle. Methylmalonyl CoA is derived from propionyl CoA which enters the pathway via degradation of several amino acids including methionine and beta oxidation of odd-chain fatty acids. While the actions of methionine synthase (which is present in the cytosol) are dependent on folic acid in addition to vitamin $B_{12}$, the actions of methylmalonyl CoA mutase (which occurs in mitochondria) are dependent only on vitamin $B_{12}$ (Rosenberg, 2008). The folic acid derivative, 5,10 methylene tetrahydrofolate is involved in DNA synthesis and repair. Thus, both vitamin $B_{12}$ and folic acid play crucial roles in the genomic stability of human cells by

preventing chromosomal breakage and hypomethylation of DNA (Fenech and Ferguson, 2001) .

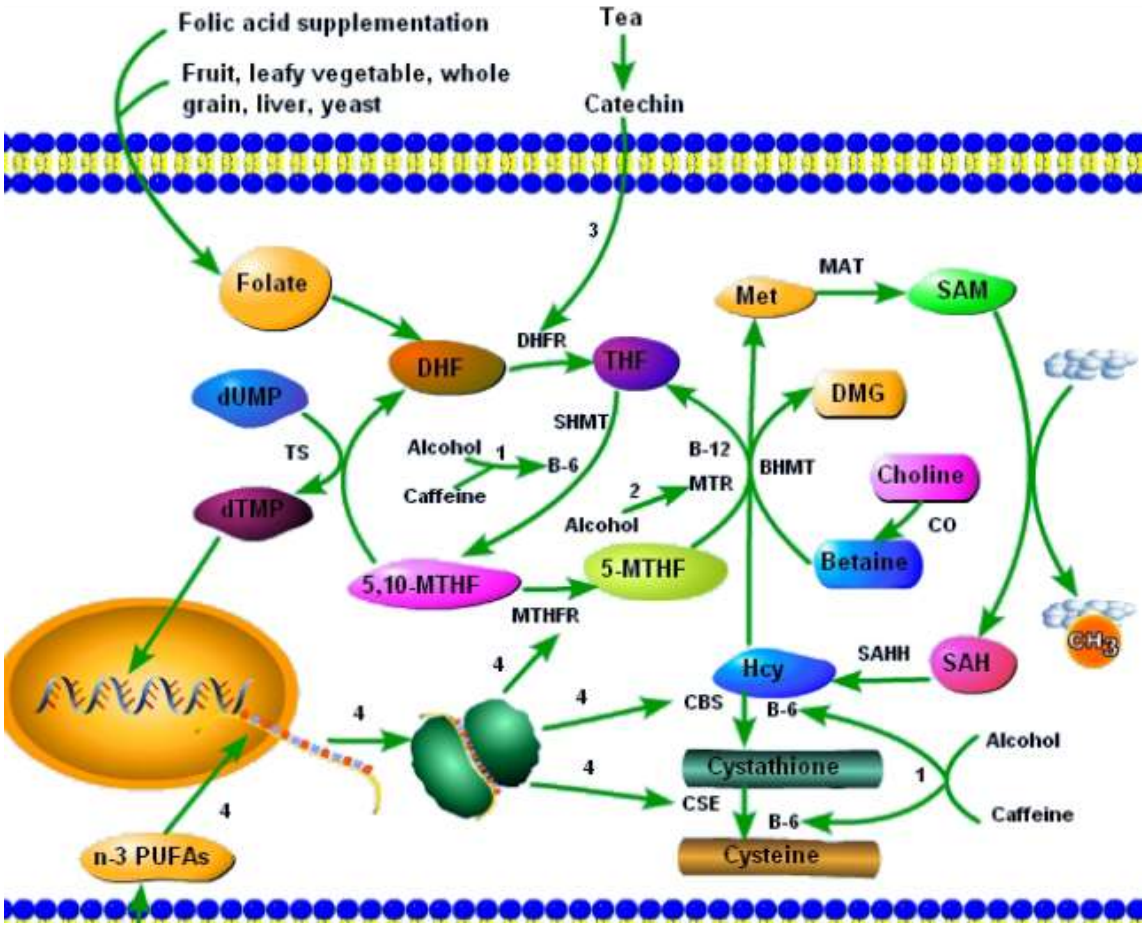


Figure 2.9. Dietary factors that affect one-carbon metabolism (Liu et al., 2013)

### 2.4.5. Insufficient Vitamin $B_{12}$ and Unfavorable Effects on Pregnancy

Cobalamin maintains natural folacin metabolism that is necessary for cell proliferation, particularly in quickly dividing placental and embryonic tissues. Along with folic acid and pyridoxine, it works as a coenzyme in DNA synthesis and many methylation reactions that happen during foetal growth (Molloy et al., 2008). Few studies have shown that the placenta synthesizes transcobalamin and includes transcobalamin receptors. Thus cobalamin and folic acid can be provided to fetus. The baby utilizes the obtainable quantities of cobalamin for biochemical interactions but is

not able to synthesize cobalamin (Molloy et al., 2008). During the third trimester of conception there is a gradual reduction in the plasma levels of vitamin $B_{12}$ due to hemodilution, hormonal alterations, changes in the levels of cobalamin binding proteins and placental transfer of vitamin $B_{12}$ to the fetus. Minimum levels of cobalamin appears at 32 weeks of conception and before birth, it rises again to reach a natural status after delivery. The concentration of the effective portion of cobalamin, holo-transcobalamin, stays unchanged through conception (Lee et al., 2009). There are no reference rates available for cobalamin levels in pregnant women. Hence, the reference rates for non-pregnant women are always utilized to assess the cobalamin levels in conception (Dror and Allen, 2012). It has been proposed that women should begin conception with plasma cobalamin concentrations of 221 pmol/L and that a concentration over 295 pmol/L is useful to reduce the danger of the growth complications (Krishnaveni et al., 2009).

Vitamin deficiencies through conception have dangerous effects on the growth of baby. However, hematological markers of cobalamin deficiency only happen in very acute shortage. Vitamin $B_{12}$ levels through conception is risky because maternal cobalamin shortage can impact the conception results for both mother and the embryo. For women who want to get pregnant, cobalamin insufficiency means increase risk of developing pre-eclampsia, intrauterine growth retardation and premature birth (Hübner et al., 2008; Krishnaveni et al., 2009). Vitamin $B_{12}$ deficiency increases the danger for low birth weight (Butler et al., 2006; Molloy et al, 2008; Krishnaveni et al., 2009). Also researches reported the relation between vitamin $B_{12}$ status and preterm births or low birth weight. Yet it was reported that 65% of pregnant women in Nepal in an urban communities had deficiency of $B_{12}$ which was linked to high total homocysteine and caused doubling preeclampsia and preterm delivery (Bjorke et al., 2001). Recent researches have also found a relationship between low cobalamin levels in mothers and neural tube defect. This leads to an increased risk of birth defects (Krishnaven et al., 2009). It might be also linked to increased obesity that might lead to insulin resistance and gestational diabetes (Saxena and Carmel, 1987; Hure et al. 2012). Also there is an extending proof that deficient or lacking vitamin $B_{12}$ situation may be linked with other complexities of conception. It was reported that the low cobalamin was linked with

conception cases impacted by anencephaly. In all studies the minimum levels of vitamin $B_{12}$ were different from 180 pg/ml to 350 pg/ml. It is not clear which minimal levels of vitamin $B_{12}$ causes the high risk of complications through conception (Schorah et al., 1980; Kirke et al., 1993).

### 2.4.6. Vitamin $B_{12}$ in Neonates and Children

Various researches have analyzed maternal and fetus/new born vitamin $B_{12}$ and a few studies confirmed practical inadequacy by using biomarkers named absolute homocysteine and MMA. Studies about maternal-umbilical line combined plasma tests demonstrate that vitamin $B_{12}$ in the fetal compartment is as twice as a mother's, plus strong positive relationship was observed between concentricity in the fetal and maternal blood circulation (Bjorke et al., 2001; Molloy et al., 2002). Besides, during childbirth the fetal total homocysteine concentration is firmly anticipated by the mother's and fetus's vitamin $B_{12}$, instead of folate (Bjorke et al., 2001; Molloy et al., 2002). In any case, plasma MMA, that is a mark of cobalamin capacity irrelevant to folate, is likewise firmly connected with cobalamin as of now, both in mothers and in newborn children (Bjorke al., 2001). A couple reports have indicated immediate rise of MMA in blood and urine of new born up to six months (Shih et al., 1976; Bjorke and Ueland, 2003), yet it stays misty what if this linked to the case of lower vitamin $B_{12}$ levels or whether different structures required in MMA treatment of that are free of vitamin $B_{12}$ limit are not totally made. Be that as it may, Bjorke et al. completed specific examination of signs of folate and vitamin $B_{12}$ situation in 700 children and young people between four days and nineteen years of age and indicated clear increments in plasma MMA and aggregate homocysteine in the initial six months of age which were emphatically connected with vitamin $B_{12}$, yet not with folic acid fixations (Bjorke et al., 2003). It should be said that there is no connection among these perceptions and newborn child hematological parameters, whilst neurological parameters were not evaluated. In any case, they suggested that there may be prevented vitamin $B_{12}$ situation in children, especially in breastfeeding infants, since condition encourages are by and large upgraded with vitamin $B_{12}$. This is steady with another information indicating great discharge of

MMA or aggregate homocysteine in newborn children who are breastfed and gives a contention to urge mothers to breast-feeding to keep up a high vitamin $B_{12}$ situation by eating regimen or supplementation (Specker et al., 1990). This is especially significant for mothers on macrobiotic eating methodologies, since it is surely understood that rigorous veggie lover weight control plans are connected with expanded danger of vitamin $B_{12}$ insufficiency (Herbert, 1994). By a wide edge the most understood explanation for babies vitamin $B_{12}$ deficient of the fact that the mother uses a strict veggie eating routine and starts conception or breast feeding with lacking tissue stores to support the requests of the embryo or neonate (Allen, 1994; Bjorke et al., 2003). Presumably the most genuine result of vitamin $B_{12}$ insufficiency amid the postpartum period is retarded growth and capacity of neurological process. Numerous vital neural processes including myelination are finished in early stages. Serious neurological impacts include the traditional demyelination lesion (subacute combined the spine cord degeneration) have been archived in newborn children of mother on eating methodologies which are inadequacy in vitamin $B_{12}$—notwithstanding the way which mother themselves won't not hint at clinical vitamin $B_{12}$ lack (Jadhav et al., 1962).

The degree and scope of conceivable outcomes coming about because of delayed peripheral inadequacy of vitamin $B_{12}$ in the postpartum phase are not very much reported, but rather it has been demonstrated that even in presence of rectification of the lack in early age, these youngsters may indicate confirmation of tenacious and long haul neurologic and subjective disability, includes crabbiness, anorexia, inability to flourish, formative relapse and poor scholarly advance (Graham et al., 1992).

## 3. CONCLUSION

Despite the fact that there is a clear indication for the negative results of insufficiencies in cobalamin and folic acid on the growth of brain amid infancy and on depression amid puberty and on fetus amid conception, neither the mechanisms, nor the effect of mild insufficiencies have been plainly determined. Both cobalamin and folate assume vital roles in the developing nervous system. Folate is essential among early fetus growth to avoid NTD and cobalamin insufficiency may affect early development during disturbances in myelination and dendritic formation or inflammation. While treatment with cobalamin may remedy some of the negative impacts of severe cobalamin deficiency on behavioral and developmental functioning, there is an indication signifying that cobalamin shortage early in life may compromise psychoeducational functioning through adolescence.

Due to propagation of $B_{12}$ deficiency in underdeveloped countries, future examinations ought to concentrate on the relation among the lacking and bordering cobalamin levels and outcomes such as congenital defects, social growth and ways to improve the cobalamin levels.

Upgrades in maternal nutritious levels can possibly enhance wellbeing results for infants. Because of the consequences of poor maternal vitamin status on the outcome of pregnancy and offspring, as a clinician it is important to pay attention to this topic when women come with a childbearing desire. On account of the results of poor maternal vitamin levels on the results of conception as a clinician it is critical to focus on this point when women accompany a childbearing need. Early analysis of $B_{12}$ and folate insufficiency before getting pregnant and prevention of maternal micronutrient insufficiency in the periconceptional period is a conceivable way to maintain a strategic distance from a few inconveniences amid conception and diminish medical issues in the ınfant.

Before starting supplementation without any proven benefit or with potential harmful effects, we require random prospective studies to decide the impact of vitamin $B_{12}$ and folate supplementary on conception and decide the ideal way and dose of administration if needed.

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