Serum and Red Cell Folate Levels and Low Birth Weight Neoborns in Wad madani Obstetrical and Gynaecological Teaching Hospital, Gezira state, Sudan (2016)

By
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February 2017
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Alfatih Khadir Ahmed abdelgadir

A Dissertation
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Department of Pathology
Faculty of Medicine

February 2017
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Serum and red cell folate Levels among low birth weight neoborns in Wad madani Obstetrical and Gynaecological Teaching Hospital, Gezira state, Sudan (2016)

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Date of examination: 23 March 2017
Dedication

This Dissertation is dedicated to:

my mother, a strong and gentle soul who taught me to trust in Allah, believe in hard work and that so much could be done with little.

My father for earning honest living for us and for supporting and encouraging me to believe in myself.
Acknowledgements

Prof. AwadAlseed Mustafa has been the ideal thesis supervisor. His sage advice, insightful criticisms, and patient encouragement aided the writing of this Dissertation in innumerable ways. I would also like to thank Dr. Alfadil Eissa, Dr. Suad fadlalah and Dr. Sulafa altayeb for their steadfast support of this dissertation which was greatly needed and deeply appreciated.
مستقبلات حمض الفوليك في المصل والخلية الحمراء في المواليد ذوي الوزن المنخفض عند الولادة في مستشفى مدني للنساء والتوليد التعليمي، ولاية الجزيرة، السودان (2016)

الفائز خضر احمد عبدالقادر

ملخص الدراسة

الملمح الأساسي في التطور الجنيني هو الانقسام المتتابع للخلايا الجنسية والتي يلعب فيها حمض الفوليك دور مركزي لدخوله في بناء وتصنيع الحمض النووي في الخلايا. أثناء نقص حمض فوليك أثناء الحمل، يُمكن أن يُضعف النمو الخلوي في الجنين أو المشيمة. نقص الوزن الولادي له علاقة وثيقة بزيادة الاعتدال والوفيات وتقلص التطور الإدراكي، وتأثراً مزمنة لاحقًا في الحياة. العديد من البحوث وجدت أن هناك علاقة مباشرة بين مستويات حمض الفوليك في الحبل السري ونقص الوزن عند الولادة في السودان لا توجد دراسات مماثلة. وقد أجريت هذه الدراسة في مستشفى ود مدني للنساء والتوليد التعليمي في الفترة بين أبريل وأكتوبر 2012. وكانت دراسة حالة هدفت لقياس مستويات حمض الفوليك وعلاقتها بالوزن عند الولادة بين مجموعتين من ذوي الوزن الولادي الطبيعي والمنخفض.

تم قياس مؤشرات الدم باستخدام عداد الخلية الآلي والفحص المجهري للطائرة الدم المحيطي والشبكات. تم قياس حمض الفوليك في المصل والخلايا الحمراء عن طريق تكنولوجيا الترهم الكيموكهرباني. تم التحليل الإحصائي بإستخدام برنامج SPSS 20 الإصدار.

إحصائيات هنالك ارتباط كبير بين مستويات حمض الفوليك في خلايا الليمفاويات 0.047 في مجموعة الوزن المنخفض. وكان ثلاثة عشر من P 0.047 المحمومات والوزن عند الولادة (قيمة من ذوي الوزن المنخفض لديهم ارتفاع في مستويات حمض الفوليك في الخلايا الحمراء و 43% من مجموع وزن المنخفض في مجموعة الفوليك بين المحمومات. الوزن المنخفض 0.05 في مجموعات حمض الفوليك في مصل الدم ووزن عند الولادة حالة (قيمة المنخفض) قد يكون هذا بسبب حقيقة أن حمض الفوليك في مصل الدم يرتبط بالحالة العضوية الاتية. مكملت حمض الفوليك خلال فترة الحمل تحد من انخفاض الوزن عند الولادة والذي يزيد من معدلات الاعتدال والوفيات على مدى الطويل.
Serum and Red Cell folate Levels among Low Birth Weight Neonates in Wad Madani Obstetrical and Gynaecological Teaching Hospital, Gezira state, Sudan (2016)

Alfatih Khadir Ahmed abdelgadir

**Abstract**

A central feature of embryonic and fetal development is widespread cell division; folate is central because of its role in nucleic acid synthesis. During gestation folate deficiency can impair cellular growth and replication in the fetus or placenta. Low birth weight is closely associated with inhibited growth and cognitive development, and chronic diseases later in life. Many researches found a relationship between umbilical cord folate status and intrauterine growth restriction. In sudan no similar study was found. This study was conducted in Wad Madani Obstetrical and Gynaecological Teaching Hospital in the period April – October 2016. It was a case-control study aimed to measure folate levels among groups of normal and low birth weight neonate to evaluate the correlation between umbilical cord folate levels and birth weight. Haematological parameters were measured using automated cell counter, microscopic examination of peripheral blood smears and reticulocytes preparations.

Serum and red cell folate were measured by electrochemiluminescence technology (cobas e 411 analyzer). Statistical analysis was done using SPSS program version 20. From the study it was found that umbilical cord RBC folate status to be an important predictor of newborn birth weight, with increasing cord RBC folate being associated with increasing newborn birth weight. Statistically significant association was found between red blood cell folate levels and birth weight (P value of 0.047 in case group). Thirteen out of 43 cases had low red cell folate levels. This indicates the presence of relationship between folate levels in fetus and birth weight. No
A statistically significant association was found between levels of serum folate and birth weight in the case group (P Value 0.59 in case group). This may be due to the fact that serum folate is a marker of recent dietary intake and is subjected to prandial variation.

Folate supplementation during pregnancy reduces the risk of low birth weight which has long-term morbidity and mortality.
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Introduction and Literature Review

1.1 Introduction

Folic acid, the oxidized form of vitamin B9, and its reduced derivative, tetrahydrofolate, are water-soluble vitamins commonly termed folates. The term folate stems from the Latin word folium which means leaf; indeed, folates are present in substantial amounts in green leafy vegetables\(^1\). Folic acid is an essential vitamin for a wide spectrum of biochemical reactions, however, unlike bacteria and plants, mammals are devoid of folate biosynthesis and thus must obtain this cofactor from exogenous sources. Therefore, folate deficiency may impair the de novo biosynthesis of purines and thymidylate and thereby disrupt DNA and RNA metabolism, homocysteine remethylation, methionine biosynthesis, and subsequent formation of S-adenosylmethionine (the universal methyl donor) which in turn may lead to altered methylation reactions. This impaired folate-dependent intracellular metabolism can lead to several key pathologies including, for example, megaloblastic anemia, homocysteinemia, cardiovascular disease, embryonic defects, in particular neural tube defects\(^1\).

As pregnancy is a period of rapid growth and cell differentiation for both mother and fetus, the demand for nutrients increase during pregnancy and approximately 20% of women show deficiency of folate and/or B12\(^2,3,4\). Several environmental risk factors such as poor nutritional status and lifestyle may lead to serious health problems in pregnant women.

Folate is important cofactor involved in homocysteine metabolism. The high serum or plasma levels of total homocysteine (tHcy) have been associated with a wide range of clinical conditions, including placental vasculopathy which have well-established effects upon fetal growth\(^5,6\). There has been increasing interest in the possible effects of folic acid supplementation during pregnancy on birth weight and the relationship between maternal folate status and birth weight has been investigated by a number of groups, most commonly in relation to intrauterine growth restriction, earlier studies relied on assessment of maternal folate intake.
reported a twofold greater risk of infant low birth weight in women with a low mean daily folate intake\(^7\).

According to World Health Organization (WHO) guidelines in 2002, about 11 million of children, aged 0–4 years, are dying every year worldwide, with a frequency of 99% in developing countries. Malnutrition is associated with more than 60% of these deaths among those very young suggest that deficient pregnant women are unable to provide the necessary amounts of several micronutrient including folate to their fetuses.

Sudan is a large country with one of the highest heterogeneous population owing to several waves of immigration, which resulted in cultural, socioeconomic and ethnic diversity among different geographic regions with different nutritional habits. The goal of the current study is to measure folate levels among groups of normal and low birth weight neonate from Wadmedani Obstetrical and Gynaecological Teaching Hospital to determine the relationship between folate level in the umbilical cord and birth weight.
1.2 Literature Review:

Folate is a water-soluble organic compound which belongs to the group of B-vitamins. It is an essential micronutrient required for the synthesis of ribo- and deoxyribonucleic acids (RNA and DNA) and consequently for cell division and tissue growth, methylation reactions and amino acid metabolism.

1.2.1 Folate chemistry

Folate is a generic term used for a group of compounds with a basic structure consisting of a pteridine residue linked through a methylene bridge to p-aminobenzoic acid, to which one or more glutamate residues are bound by peptide bonds. The pterin moiety exists in three oxidation states (oxidised, partially reduced as 7,8-dihydrofolate and fully reduced as 5,6,7,8-tetrahydrofolate) and can be substituted at the N-5 or N-10 position by different one-carbon units\(^8\).

Figure 1.1 basic structure of folate.
Tetrahydrofolate (THF) which is the fully reduced form of the vitamin, carries one-carbon units at one of three different oxidation levels ranging from methanol to formate. In the cell, five different one-carbon substituted forms of THF are present: 10-formyl-THF; 5-formyl-THF; 5,10-methenyl-THF; 5,10-methylene-THF and 5-methyl-THF, and each of these forms is interconverted in the cell through enzyme-mediated catalysis.

In the body, the addition of glutamate residues to the monoglutamate form increases the affinity of folate cofactors for folate-dependent enzymes and is required to retain folates within the cell and subcellular organelles.

Naturally occurring food folates are reduced vitamins which are usually polyglutamates containing five to seven glutamate residues.

Natural folates are unstable and some losses occur in the presence of light and oxygen and at high temperatures.

In contrast, folic acid, one of the synthetic forms of the vitamin, is a fully oxidised monogluta
mate and is the most chemically stable form. However, folic acid is not a natural component of the diet and is consumed via fortified foods or food supplements only it has vitamin activity after it has been fully reduced \(^9\).

Most foods contain some folate. The highest concentrations are found in liver and yeast ( > 200 µg per 100 g), green vegetables and nuts ( > 100 µg per 100 g). Folate is easily destroyed by heating, particularly in large volumes of water; over 90% may be lost.

1.2.2 Functions of folate:

Folate functions as a cofactor or cosubstrate in numerous one-carbon transfer reactions that are important for the synthesis of RNA and DNA, amino acid interconversions and the process of methylation. Different folate forms are involved in specific reactions, but all of them are finally metabolised to THF.

1.2.2.1 Biochemical functions
Folate is essential for the synthesis of RNA and DNA and consequently for cell division and tissue growth.

The 10-formyl-THF form provides one-carbon units for the formation of purine nucleotides (adenine and guanine) that are necessary for both RNA and DNA synthesis, whereas 5,10-methylene THF is a cofactor in the reaction that generates thymidine monophosphate, a pyrimidine nucleotide specific for DNA.

Folate deficiency impairs DNA replication and cell division, which adversely affects rapidly proliferating tissues such as bone marrow and results in decreased production of blood cells.

It has also been reported that folate deficiency is associated with structural damage of DNA as a consequence of misincorporation of uracil instead of thymine, which might have implications for cancer development \(^{(10)}\).

Folate is fundamental for the normal functioning of the methionine cycle, which is responsible for both the conversion of homocysteine to methionine and the production of the universal methyl donor S-adenosylmethionine (SAM).

Folate in the form of 5-methyl-THF acts as a cosubstrate in the remethylation of homocysteine to methionine in a reaction catalysed by the enzyme methionine synthase, which also requires methylcobalamin as a cofactor.

This is an effective way to restore the essential amino acid methionine, which is used not only for protein synthesis but also for the generation of SAM. In turn, SAM donates its methyl group to more than 100 methyltransferases for a wide range of substrates such as DNA, hormones, proteins, neurotransmitters and membrane phospholipids\(^{(11)}\), all of which are regulators of important physiological processes.

As a result of this reaction, SAM is converted to S-adenosylhomocysteine and homocysteine. Folate deficiency disturbs the normal function of the methionine cycle, which results in elevation of plasma total homocysteine\(^{(12)}\) and insufficient SAM production with potential impairment of some methylation pathways. For example, reduced global DNA methylation has been reported in
1.2.3 Physiology and metabolism

1.2.3.1 Intestinal absorption:

Steps involved during intestinal absorption are active and saturable, as well as passive and unsaturable, mechanisms are involved in folate absorption.

Upon ingestion of polyglutamated forms, hydrolysis to their monoglutamates is required by γ-glutamyl carboxypeptidase (also known as folate conjugase, γ-glutamyl hydrolase or glutamate carboxypeptidase II, among others), which is located primarily in the jejunal brush border membrane(15,16).

Subsequently, a folate carrier with a similar affinity for both folic acid and reduced folate forms is involved in the transport of monoglutamates across the brush border membrane. After entering the intestinal cells, folates are usually reduced and methylated and this is followed by a carrier-mediated mechanism exporting the methyl-THF into the bloodstream, although there is also evidence that folic acid enters the portal vein unchanged, with reduction and methylation taking place only once it reaches the liver(17,18).

This active absorption mechanism is pH dependent(19) (optimal activity of γ-glutamyl carboxypeptidase at pH 6–7 and of intestinal absorption at pH ~ 5).

The body has a limited ability to convert ingested folic acid into reduced folate derivatives and, when the capacity for reduction and methylation of folic acid is exceeded, unmetabolised folic acid may appear in serum (20,21,22). In contrast, the activity of human jejunal brush border γ-glutamyl carboxypeptidase does not seem to be rate-limiting in the absorption process within the range of usual dietary intakes (23).

For folates not absorbed in the jejunum, unspecific folate absorption takes place predominantly in the ileum involving passive diffusion in linear proportion to the amount reaching the ileum.
There is also evidence from human studies with isotopically labelled folate ([13C5]5-formyl-THF) that some folate is absorbed in the large intestine\(^{(24,25)}\).

Factors influencing intestinal absorption includes incomplete release of folates from plant cellular structures may lower folate bioavailability from plant foods. Whether some types of dietary fibre (e.g. wheat bran) lower folate absorption is unclear, and many types of fibre appear not to reduce folate absorption\(^{(26)}\).

Results of in vitro studies mimicking intestinal digestion of folate from different foods indicated that food folate losses occur during digestion which may contribute to the incomplete bioavailability of food folate\(^{(27)}\).

It has been suggested that the presence of components with antioxidative properties, such as ascorbic acid, may enhance the stability of reduced folates in the digestive tract, as shown in vitro\(^{(27)}\), and that the addition of milk to the diet may enhance folate bioavailability, as shown in in vivo and in vitro studies\(^{(28)}\).

### 1.2.3.2 Transport in blood

The predominant form of folate in the circulation is 5-methyl-THF monoglutamate. It is mainly bound to albumin, which is a low-affinity folate-binding protein (about 50% of all bound folate).

However in folate deficiency, a higher proportion of folate in plasma is bound to albumin\(^{(29)}\).

Plasma also contains a soluble form of the folate receptor which binds a small proportion of folate; however, in pregnancy, its concentration is increased\(^{(29)}\).

One-third of folate in plasma is in a free form. The role of both specific and non-specific binding proteins in plasma is unclear, but it is believed that they do not have a major influence on tissue folate uptake.

After folate ingestion, plasma concentration increases and is maintained at an elevated concentration for up to approximately four hours followed by a rapid decrease\(^{(30)}\).

### 1.2.3.3 Distribution to tissues
Folate is delivered to the tissues against a concentration gradient, an energy-dependent process which requires the involvement of folate transporters (reduced folate carrier, proton-coupled folate transporter and folate receptors).

The pattern of internalisation of folate is tissue and cell specific and depends on the efficiency of the folate transporters and the cellular concentration of folate\textsuperscript{(31)}.

Once absorbed through the duodenum and jejunum, folate monoglutamates are transferred via portal circulation to the liver, where they are retained or released back into the circulation for distribution to other tissues. In order to be retained by the cells, folate monoglutamates are converted to polyglutamates by the enzyme folylpolyglutamate synthase (also termed tetrahydrofolate synthase).

The main form of folate entering the cells from the blood, 5-methyl-THF, is a very poor substrate for this enzyme\textsuperscript{(32)}; thus, it is converted to THF through a reaction involving the cobalamin-dependent enzyme methionine synthase. THF has a high affinity for folylpolyglutamate synthase and after polyglutamation can be retained by the cells. However polyglutamated folate is not only a storage form of folate in tissues, but also a functional form of the vitamin because only derivatives of folate polyglutamates are able to act as cofactors in folate dependent enzyme reactions; therefore, polyglutamation is required both for retaining folate within the cells and for the normal function of one-carbon metabolism\textsuperscript{(32)}.

Any folate which is not converted to polyglutamate is eliminated from the cells. Mature red blood cells do not have mechanisms to transport folate and the folate that they contain is accumulated only during erythropoiesis.

Placenta has the ability to concentrate folates owing to the abundance of folate receptors predominantly folate receptor-$\alpha$, reduced folate carrier and proton-coupled folate transporter in this tissue\textsuperscript{(33,34,35)}. This mechanism of folate transport across the placenta is established within the first trimester of pregnancy to satisfy the high requirements for folate during fetal development\textsuperscript{(35)}. As a result of the high folate concentration in the intervillous blood, folate in fetal blood is two to four times higher than in maternal blood\textsuperscript{(36)}.

1.2.3.4 Storage
The ability of tissues to store folates in excess of the amounts required for normal metabolism is limited \(^{(37)}\).

The exact amount of total body folate content in adults is not precisely known, and estimates range from around 22 to 100 mg \(^{(38,39,40)}\).

It is estimated that 99 % of total body folate is in the tissues \(^{(40)}\), with storage taking place predominantly in the liver \(^{(41)}\).

There is strong compartmentalisation of folate within the cell. The following three distinctive folate compartments are identified: cytosolic, mitochondrial, and nuclear. Up to 50 % of folate in the cell is in the mitochondria, predominantly in the form of 10-formyl-THF, whereas the cytosol contains mainly methyl-THF \(^{(30)}\).

1.2.3.5 Metabolism

The three folate compartments within the cell have specialised metabolic functions but, at the same time, they are interdependent, as they rely on the exchange of different metabolites \(^{(30,42)}\).

(1) Folate in the mitochondria is involved in the catabolism of serine and glycine generating formate which in turn is utilised in the cytoplasm for the remethylation of homocysteine to methionine and for the synthesis of nucleotides.  

(2) Folate in the nuclear compartment is responsible for the production of thymidylate for DNA synthesis.

(3) Folates which are not bound to specific and non-specific binding proteins are subjected to catabolism by oxidative cleavage at the C9–N10 bond, generating p-aminobenzoylglutamates which in turn are acetylated in the liver before excretion \(^{(30)}\). The whole-body turnover rate of folate is estimated to be 1 % of body folate pools \(^{(43)}\).

1.2.3.5.1 Disorders of metabolism
A number of infants have been described with congenital defects of folate enzymes e.g. orotic aciduria which is refers to an excessive excretion of orotic acid in urine. It causes megaloblastic anemia and may be associated with mental and physical retardation. It can be caused by a deficiency in the enzyme Uridine monophosphate synthetase; a bifunctional protein that includes the enzyme activities of orotate phosphoribosyl transferase and orotidine 5'-phosphate decarboxylase.

It can also arise secondary to blockage of the urea cycle, particularly in ornithine transcarbamylase deficiency.

Orotic aciduria is associated with megaloblastic anaemia due to decreased pyrimidine synthesis.

1.2.3.6 Elimination

Folate is filtered through the renal glomeruli but most of it is reabsorbed in the proximal tubule with the assistance of folate-binding proteins and specific transporters\(^{(44)}\). As a result, most of the folate in the urine is in the form of breakdown products, with only 1–2 % of the excreted amount being active folate\(^{(45,46)}\).

The majority of faecal folate is synthesised by intestinal microorganisms; however, the loss of endogenous folate (biliary folate together with folate from shedded intestinal cells) also occurs via this route.

During lactation, folate is secreted via breast milk, where it is bound to folate-binding proteins. The presence of folate-binding proteins in mammary gland tissue facilitates folate uptake from the circulation, since milk folate concentration is typically 5–10 times higher than that of maternal plasma\(^{(47,48)}\).

1.2.3.7 Interaction with other nutrients
Folate interacts with cobalamin in one of the key reactions in the methionine cycle. Cobalamin functions as a cofactor and 5-methyl-THF acts as a cosubstrate for the enzyme methionine synthase, the main role of which is to remethylate homocysteine to methionine for a subsequent production of SAM required for the methylation of various substrates\(^{(49)}\).

Another important function of the methionine synthase reaction is to convert 5-methyl-THF to THF which is used for polyglutamation (THF rather than 5-methyl-THF is a preferable substrate for folylpolyglutamate synthase) for nucleotide synthesis. Therefore, cobalamin has a critical role in both the retention of folates in tissues and the provision of folate-derived one-carbon units for DNA synthesis or for methylation reactions. In cobalamin deficiency, the methionine synthase reaction is reduced and 5-methyl-THF is trapped in this form, since it cannot be metabolized in

Clinically, the condition may manifest by haematological and neurological abnormalities, but its distinctive metabolic features include high serum folate concentration in combination with low red blood cell folate concentration and high total homocysteine concentration \(^{(52,55)}\).

Vitamin B6 in the form of pyridoxal 5’-phosphate acts as a cofactor for the enzymes hydroxymethyltransferase and glycine decarboxylase, which transfer one-carbon units from serine and glycine, respectively, for the generation of 5,10-methylene-THF in the cytoplasm and mitochondria. These reactions are critical for the normal function of the folate and methionine cycles.

1.2.3.8 Biomarkers

1.2.3.8.1 Biomarkers of intake and status
Serum folate concentration

Folate concentration measured in serum or plasma is considered to be a sensitive marker of recent dietary intake and it is subjected to prandial variation \(^{(56)}\). However, a single measurement of serum/plasma folate is not very informative for the assessment of folate status and body stores \(^{(56)}\).

A single measurement of serum/plasma folate reflects only the time of blood collection and cannot differentiate between occasional low dietary intake of the vitamin and folate deficiency. Therefore, in order to obtain information on folate status, a single measurement of serum folate should be combined with other biomarkers of folate status. The combined use of plasma and red blood cell folate for the assessment of folate status of populations was recommended by a World Health Organization (WHO) \(^{(57)}\).

Red blood cell folate concentration

Red blood cell folate is considered the most reliable biomarker of folate status, as it reflects tissue folate stores \(^{(58)}\). Folate is incorporated into red blood cells only during their maturation in the bone marrow and folate concentration remains stable throughout the 120-day lifespan of the cells. Red blood cell folate is an indicator of long-term folate status and decreases only months after the initial reduction of folate intake and the fall in serum folate concentration.

1.2.3.8.2 Biomarkers of function

Plasma total homocysteine

In the methionine cycle, folate cofactors are involved in the remethylation of homocysteine to methionine. Plasma total homocysteine concentration is used as a biomarker of folate function. However, plasma total homocysteine is not specific for folate function, since it is also affected by other B-vitamins (cobalamin, vitamin B6 and riboflavin).
-Mean cell volume

Macrocytic cells appear in the bone marrow shortly after initiation of folate depletion and before the fall in red blood cell folate concentration\(^{(59)}\).

However, given the long lifespan of the circulating red blood cells (i.e. 120 days) in the peripheral blood, macrocytosis can be detected at only an advanced stage of folate deficiency \(^{(60)}\).

### 1.2.4 Causes of folate deficiency

**Impaired absorption:** Due to decreased duodenal and ileal absorption of folate, intestinal dysfunction (Crohn’s disease, Celiac disease), congenital abnormality in intestinal folate transporter and medication that affect folate metabolism or possibly absorption (e.g., methotrexate, phenytoin, carbamazepine).

**Insufficient dietary intake:** Due to Poor nutrition.

**Increased requirements:** as a result of increased cellular proliferation as in pregnancy, lactation, haemolytic anemia (e.g. sickle cell anemia), malignancies (associated with a high proliferative rate) and exfoliative dermatitis.

### 1.2.5 Folate and pregnancy outcome

Folate plays a crucial role in the one-carbon metabolism for physiological nucleic acid synthesis and cell division, regulation of gene expression, amino acid metabolism and neurotransmitter synthesis.

During pregnancy, increased folate intake is required for rapid cell proliferation and tissue growth of the uterus, placenta and growth of the fetus.
A central feature of embryonic and fetal development is widespread cell division; folate is central because of its role in nucleic acid synthesis. During gestation, folate deficiency can impair cellular growth and replication in the fetus or placenta.

During pregnancy, low concentrations of dietary and circulating folate are associated with increased risks of infant low birth weight (<2500 g), preterm delivery (<37 wk gestation), and fetal growth retardation.

A metabolic effect of folate deficiency is an elevation of blood homocysteine. The high serum or plasma levels of total homocysteine have been associated with a wide range of clinical conditions, including placental vasculopathy which have well-established effects upon fetal growth. Likewise, the presence of maternal homocysteine concentrations have been associated both with increased habitual spontaneous abortion and pregnancy complications (eg, placental abruption and preeclampsia), which increase the risk of poor pregnancy outcome and of decreased birth weight and gestation duration.

The relationship between folate status and birth weight has been investigated by a number of groups, most commonly in relation to intrauterine growth restriction.

In a study about the vitamin status in low birth weight neonate J. Novaro et al. demonstrate clear association between folate deficiency and low birth weight neonate.

Rondo et al reported that more than 25.7% of the intrauterine growth restricted newborn had low cord RBCs folate levels.

Hong Seok et al reported a significant correlation between birth weight and folate level of umbilical cord.

2.1 Justification

During pregnancy, low concentrations of circulating folate are associated with increased risks of infant low birth weight.
Low birth weight is closely associated with fetal and neonatal mortality and morbidity, inhibited growth and cognitive development, and chronic diseases later in life.

Detection of incidence of folate deficiency in low birth weight neonate increases the awareness of the role of folate supplementation during pregnancy thus reducing fetal and neonatal mortality and morbidity.

2.2 Objectives:

2.2.1 General objective:-

To study the prevalence of folate deficiency among low birth weight neonates delivered in Wad medani Obstetric and Gynaecological Teaching Hospital from April to October 2016.

2.2.2 Specific objectives:

- To measure the prevalence of folate deficiency among low birth weight neonate.

- To measure the serum folate in low birth weight neonate using 
  Electrochemiluminescence technology.

- To measure the red cell folate in low birth weight neonate using 
  Electrochemiluminscence technology.

- To determine the association between folate levels and infant birth weight.

Materials and Methods:

3.1. Study design:

This was a Case-control study conducted in Wad Medani Obstetrical and Gynaecological Teaching in the period from april 2016 to October 2016.
3.2 Study area:

Wad Medani Obstetric and Gynaecological Teaching Hospital, Wad Medani locality, Gezira State. It is the first specialized hospital in Obstetric and Gynaecology in Gezira state and second one in Sudan. It involves emergency unit, refer clinic, delivery section, theatre, intensive care unit, neonatology unit, short and long stay ward, pharmacy, and employee offices. The hospital capacity is 332 beds.

The neonatology unit provides care of ill and premature newborn, directed by two paediatricians and staffed by nurses.

3.3 Study setting:

Medical laboratory, Gezira University and National institute of cancer.

3.4 Study population:

Normal and low birth weight newborn delivered in Wad medani Obstetric and Gynaecological Teaching Hospital during the study period.

3.4.1 Inclusion criteria:

Low birth weight neonates and normal control delivered in Wad medani Obstetric and Gynaecological Teaching Hospital during the study period.

3.4.2 Exclusion criteria:

- Exclusion of other causes of low birth weight newborn.
- Congenitally malformed newborn.

3.4.3 Sample size:

86 normal and low birth weight neonate.

\[ n = \frac{Z^2 \cdot p \cdot Q}{d^2} \]

\( n = \) sample size

\( d = \) the degree of accuracy desired or the margin of error tolerated

\[ Z^2 = \text{confident score} = 1.96 = 2 \]

\( P = \) is proportion of the target population estimated to have a particular characteristic \( Q = 1 - P \)
3.5 Data collection tools:

Methods: Eighty six newborn delivered in Wad Medani Obstetric and Gynaecological Teaching Hospital during the study period were consecutively enrolled for the study. A medical history from the mothers and physical examination of the neonate were performed. Blood samples were drawn from the umbilical cord for Complete Blood Count (CBC) and for measuring serum and red cell folate levels. Data was collected by questionnaire including:

1. Personal data;
2. History
3. Physical examinations to look for pallor, congenital malformation and to measure birth weight.
4. Investigations: Blood was collected in EDTA anticoagulant to perform CBC, peripheral blood smears and reticulocyte count. Electrochemiluminescence immunoassay to measure serum and red cell folate.

3.5.1 Sample collection:
Complete blood count, peripheral blood smears and reticulocyte preparations were done from umbilical cord blood samples. Samples were taken from umbilical vein by a 5 ml syringe. Umbilical cord blood was taken into three containers:
(1) 2.5ml was taken in K3 EDTA containing tubes for measuring Complete Blood Count (CBC).
(2) 2.5 ml was obtained in K3 EDTA containing tubes and stored at -20°C for measuring red cell folate.
(3) 3ml was obtained in plain tubes. The samples were centrifuged for 5 minutes and serum was taken in another 2.5 plain tubes. These sample were stored at -20°C till tested.

CBC was done using an automated blood cell counters (sysmex KN21 analyzer) with a flow cytometry using a laser light to perform full blood count haemoglobin concentration (Hb), red blood cells count (RBCs) haemotocrit (Hct), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood counts (WBCs) and platelet counts (PLTs).

3.5.2 Making a blood film:-
Manual spreading of blood films using frosted glass slides were performed.
The frosted glass slides were cleaned. A drop of blood was placed near one end of the slide and spreader was applied at an angle of 45°, in front of the drop of blood making a thin blood film and allowed to dry. Then they were labeled with the sample number. The films were placed horizontally on the staining rack and flooded with Leishman's stain and left for 5 minutes. A double volume buffer was added with gentle blowing over the surface without touching the film surface. The films were left for another 10 minutes and then washed off with tap water. The back of the slides was cleaned using cotton dipped in alcohol and then left to dry.

3.5.3 Examination of the blood films:
The identification of the specimen was checked and matched with the Corresponding complete blood count (FBC) report. Then films were examined microscopically using a low power field (10 objectives) and (100 objectives) to determine the suitable area for blood film examination. The morphology of the red cells regarding the staining character, shape, size of the cells and the presence of nucleated red blood cells.
The White blood cells and platelets were examined and assessment of their number, size, morphology and presence of aggregate of platelets were also evaluated. Correction of the TWBC was applied when necessary (presence of NRBCs).

3.5.4 Reticulocytes: A reticulocyte preparation was done by adding 2 drops of blood to 4 drops of methylene blue stain. After mixing the mixture is incubated at 37°c in a water path for 15 minutes. A drop is taken and spread in a glass slide, allowed to dry and examined microscopically by the 100 oil emersion to count the percentage of the reticulocytes.

3.5.5 Electrochemiluminescence immunoassay
Serum and Red cell folate levels were measured using electrochemiluminescence immunoassay (Roche Laboratories, Mannheim, Germany).
Based on this technology and combined with well-designed, specific and sensitive immunoassays it provides superior performance reliable results.

3.5.5.1 Measurement of serum folate

Test principle:

![Diagram of test principle](image)

**Figure 3.1 Test principle (Competition principle).**

Competition principle. Total duration of assay: 27 minutes

First incubation: By incubating 25 µL of sample with the folate pretreatment reagents 1 and 2, bound folate is released from endogenous folate binding proteins.

Second incubation: By incubating the pretreated sample with the ruthenium labeled folate binding protein (antibody), a folate complex is formed, the amount of which is dependent upon the analyte concentration in the sample.

Third incubation: After addition of streptavidin-coated microparticles and folate labeled with biotin, the unbound sites of the ruthenium labeled folate binding protein become occupied, with formation of a ruthenium labeled folate binding protein-folate biotin complex. The entire complex becomes bound to the solid phase via interaction of biotin and Streptavidin.

The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then
removed. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

**Figure 3.2: Reaction phase of electrochemiluminescence immunoassay**

**Calculation:**

The analyzer automatically calculates the analyte concentration of each sample (either in nmol/L or ng/mL).

Conversion factors: $\text{nmol/L} \times 0.44 = \text{ng/mL}$.

$\text{ng/mL} \times 2.27 = \text{nmol/L}$.

Serum folate range:

5.3-19.3ng/ml

3.5.5.2 Measurement of Red cell folate

**Test principle:**
Competition principle, Total duration of assay: 27 minutes.

Whole blood treated with anticoagulants (heparin or EDTA) is mixed with ascorbic acid solution and incubated for approximately 90 minutes at 20-25 °C. Lysis of the erythrocytes takes place, with liberation and stabilization of the intracellular folate. The resulting hemolysate sample is then used for subsequent measurement.

Incubation and measurement follow the same principle that used for measuring Serum folate.

To calculate the folate concentration in the erythrocyte fraction of the sample (RBC folate), the predetermined sample specific hematocrit value must be taken into account using the following equation:

\[
\text{RBC folate} = \frac{\text{analyzer result}}{\text{haematocrit}} \times 100
\]

RBC folate range:
629 – 1453 ng/mL.

Reagents - working solutions:

The reagent rackpack (M, R1, R2) and the pretreatment reagents (PT1,PT2) are labeled as Fol III.

PT1: Pretreatment reagent 1, 1 bottle, 4 mL Sodium 2-mercaptoethanesulfonate (MESNA) 40 g/L, pH 5.5.

PT2: Pretreatment reagent 2, 1 bottle, 5 mL Sodium hydroxide 25 g/L.

M: Streptavidin-coated microparticles, 1 bottle 6.5 ml:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1: Folate binding protein–Ru(bpy), 1 bottle, 9 mL Ruthenium labeled folate binding protein 75 µg/L; human serum albumin(stabilizer) ; borate/phosphate/citrate buffer 70 mmol/L pH 5.5; preservative.
R2: Folate~biotin, 1 bottle, 8 mL. Biotinylated folate 17 μg/L; biotin 120 μg/L; human serum albumin (stabilizer); borate buffer 100 mmol/L, pH 9.0; preservative.

3.6 Data analysis:

Data was analyzed by computer software program SPSS version 20.

3.7 Ethical clearance:

Ethical clearance was obtained from the University of Gezira ethical committee and the ministry of health in Gezira state authority. Verbal informed consent was obtained from all candidates.
Results

This case control study was conducted at Wad Madani Obstetric and Gynaecological Teaching Hospital in the period from April to October 2016. The objectives of the study were to measure serum and red cell folate in the umbilical cord blood of normal and low birth weight neonate to establish the relation between folate levels and birth weight. The measurement of serum and red cell folate levels were obtained by electrochemiluminescence immunoassay.

Haematological parameters were assessed by complete blood count; peripheral blood smears examination and reticulocyte count.

A total of 86 neonate were enrolled to the study.

4.1 Descriptive statistics:

4.1.1 Concerning gender distribution

In control group (normal birth weight neonborns) there were 25 females and 18 males while in case group (low birth weight neonborns) there were 31 females and 12 males.

4.1.2 Weight and weight for age percentile:

The weight of control group ranging from 2.5 to 4.2 kg (from 3rd centile to 75th centile) while the case group was less than 2.5 kg (below 3rd centile). The mean value of birth weight of control group was found to be 3.0372 kg and the standard deviation was 0.49475 with maximum value 4.20 kg and minimum value 2 kg. The mean value of birth weight of case group was found to be 2.0326 kg and the standard deviation was 0.24950 with maximum value 2.40 kg and minimum value 1.50 kg.
4.1.3 Level of mother education:

In control group 3 mothers were illiterate, 8 were able to read and write, 15 were reached the secondary school and 17 were finished the university, while in case group 6 mothers were illiterate, 12 were able to read and write, 18 were reached the secondary school and 7 were finished the university.

4.1.4 Folic acid intake during pregnancy:

All mothers in control group received folate, 39 received folate from the 1st trimester, 3 from the 2nd trimester and 1 during the 3rd trimester, while 5 mothers in case group were not received folate during pregnancy, 32 received folate from the 1st trimester, 5 from the 2nd trimester and 1 during the 3rd trimester.

4.1.5 Anaemia during pregnancy:

1 out of 43 mothers from control group have anaemia during pregnancy while 7 out of 43 mothers in cases group developed anaemia during pregnancy, one of them transfused during the current pregnancy.

4.1.6 Other pregnancy comorbidity:

Control group: 12 mothers developed malaria during pregnancy, 4 mothers developed diabetes, 2 mothers developed hypertension and 1 mother had deep vein thrombosis during pregnancy.

Case group: 14 mothers developed malaria during pregnancy.

4.1.7 Past history of low birth weight neonates:

Control group: 3 mothers had a past history of low birth weight neonate

Case group: 7 mothers had past history of low birth weight neonate.
4.1.8 Past history of neural tube defect:

There was no past history of neural tube defect in both control and case group.

4.1.9 History of blood transfusion during pregnancy:

Control group: no history of blood transfusion in control group.

Case group: 1 mother had a history of blood transfusion during the current pregnancy.

4.1.10 History of regular antenatal care:

Control group: 3 mothers out of 43 were not on regular antenatal care during the current pregnancy.

Case group: 16 mothers out of 43 were not on regular antenatal care during the current pregnancy.

4.1.11 Intake of green leafy vegetables and meat:

Control group: 1 mother out of 43 did not eat green leafy vegetable and meat.

Case group: 9 mothers out of 43 did not eat green leafy vegetable and meat.

4.1.12 Gestational age

Control group: All neonates in control group were term.

Case group: 6 out of 43 newborn were preterm.

5 out of the 6 preterm had low red blood cell folate level.

4.1.12 CBC

Haematological parameters were measured using automated cell counter (sysmex KN 21). The various haematological parameters including white blood cell count, red blood cell count, platelet count, haemoglobin concentration, haematocrit, mean cell volume, mean cell haemoglobin and mean cell haemoglobin concentration were performed.
4.1.12-1 The white cells count mean value of the control group was found to be 12.8791 and the standard deviation was 4.34591 with a minimum value 10.4×10⁹/dl and maximum value 33.30×10⁹/dl. 42 cases were more than 10×10⁹/dl and one case 33.3×10⁹/dl. The white cells count mean value of the case group was found to be 12.0116 and the standard deviation was 2.95459 with a minimum value 7.00×10⁹/dl and maximum value 18.40. Ten cases were less than 10×10⁹/dl and 33 cases were more than 10×10⁹/dl.

4.1.12-2 The mean total red blood cell count of control group was found to be 4.1221 and the standard deviation was 0.46851 with maximum value 5.20×10¹²/l and minimum value 2.80×10¹²/l with 42 cases (97.7%) < 5×10¹²/l and one case (2.3%) 5.2×10¹²/l. The mean total red blood cell count of the case group was found to be 4.3300 and the standard deviation was 0.47085 with maximum value 5.30×10¹²/l and minimum value 3.30×10¹²/l with 39 cases (90.7%) < 5×10¹²/l and four cases (9.3%) 5–7×10¹²/l.

4.1.12-3 The mean haemoglobin level of the control group was found to be 15.2744 g/dl and the standard deviation was 1.69300 with maximum value 18.90 g/dl and minimum value 11.50 g/dl. There were 5 cases <14 g/dl and 38 cases in the ranged from 14-22 g/dl.

The mean haemoglobin level of the case group was found to be 14.6372 g/dl and the standard deviation was 1.94863 with maximum value 17.60 g/dl and minimum value 11.00 g/dl. There were 15 cases <14 g/dl and 28 cases in the ranged from 14-22 g/dl.

4.1.12-4 The mean hematocrit or packed cell volume of the control group was found to be 44.3288 and the standard deviation was 4.33378 with maximum value 53.00 and minimum value 34.70. There were 24 case <45% and 19 cases in the ranged from 45-75%.

The mean hematocrit or packed cell volume of the case group was found to be 43.9674 and the standard deviation was 5.00931 with maximum value 55.50 and minimum value 33.60 there
were 28 cases <45\% and 15 cases in the ranged from 45-75\%.

4.1.12-5 The mean level of mean corpuscular volume of the control group was found to be 101.0558 fl and the standard deviation was 4.53506 with maximum value 111.50 fl and minimum value 88.90 fl. 26 cases ranged from 100-120 fl and 17 cases were less than 100 fl.

The mean level of mean corpuscular volume of the case group was found to be 102.7764 fl and the standard deviation was 5.82654 with maximum value 122.1 fl and minimum value 84.50 fl. 29 cases ranged from 100-120 fl and 12 cases were less than 100 fl and 5 cases more than 120 fl.

4.1.12-6 The mean level of mean corpuscular hemoglobin of control group was 34.6116 pg, and the standard deviation was 1.85847 with maximum value 38.70 pg and minimum value 30.10 pg.

The mean level of mean corpuscular hemoglobin of case group was 34.0698 pg, and the standard deviation was 1.92793 with maximum value 37.40 pg and minimum value 27.60 pg.

4.1.12-7 The mean level of mean corpuscular hemoglobin concentration of control group was 34.3116 and the standard deviation was 0.91787 with maximum value 36.30 g/dl and minimum value 31.30 g/dl.

The mean level of mean corpuscular hemoglobin concentration of case group was 33.8116 and the standard deviation was 0.73199 with maximum value 35.70 g/dl and minimum value 32.50 g/dl.

4.1.12-8 The mean level platelet count of the control group was found to be 235.3721 and the standard deviation was 76.44980 with maximum value 438.00 and minimum value 57.00. 39 cases ranged from 100-450 and 2 cases were less than 100.

The mean level platelet count of the case group was found to be 247.8140 and the standard deviation was 85.98983 with maximum value 436.00 and minimum value 48.00. 39 cases ranged from 100-450 and 4 cases were less than 100.

4.1.13 Microscopic examination:

4.1.13-1 Red blood cells
Control group:

Anisocytosis was observed in 32 cases (74.4%), normocytic cells were seen in 11 cases (25.6%).
   No rouleaux formation or autoagglutination noticed.

Mild hypochromia was noticed in 10 cases (23.3%) and 33 cases (76.7%) were normochronic.
   Nucleated red blood cells were seen in 32 cases (74.4%).

Case group:

Anisocytosis was observed in 23 cases (53.5%), normocytic cells were seen in 13 cases (30.2%)
   macrocytes in 7 cases (16.3%). No rouleaux formation or autoagglutination noticed.

Mild hypochromia was noticed in 4 cases (9.3%) and 39 cases (90.7%) were normochronic.
   Nucleated red blood cells were seen in 35 cases (81.4%).

4.1.13.2 White blood cells

Control group:

Reactive lymphocytes was observed in 1 case (2.3%) and left shift was observed in one case (2.3 %).

Case group:

Hypersegmented neutrophils were observed in 9 cases (20.9%) and reactive lymphocytes in 2 cases (4.7%).

4.1.13.3 Platelets:

Control group:

Platelet aggregates were seen in 1 case and 1 case had giant forms.
Case group:

Giant forms were observed in 2 cases.

4.1.14 Reticulocytes

Control group:

The mean reticulocytes count was 1.0209 with 0.59344 standard deviation. The minimum value was 0.2% while the maximum reticulocyte count was 2.8%.

Case group:

The mean reticulocytes count was 0.9535 with 0.59815 standard deviation. The minimum value was 0.2% while the maximum reticulocyte count was 2.6%.

4.1.15 Folate level

4.1.15.1 Serum folate level

Control group:

The mean serum folate level of control group was 12.3859 and the standard deviation was 3.18918 with maximum value 20.0 ng/ml and minimum value 8.20 ng/ml.

Case group:

The mean serum folate level of control group was 10.6037 and the standard deviation was 3.30492 with maximum value 18.01 ng/ml and minimum value 4.4 ng/ml.

4.1.15.2 Red cell folate level

Control group: The mean red cell folate level of control group was 1380.3651 and the standard deviation was 69.73678 with maximum value 1550 ng/ml and minimum value 1302 ng/ml.

Case group: The mean red cell folate level of control group was 930.0428 and the standard deviation was 226.93620 with maximum value 1345 ng/ml and minimum value 500 ng/ml.
4.2 Inferential statistic

4.2.1 Cross tabulations

Cross tabulation was done to detect associations between folate level and birth weight, RBCs folate level was found to be related to birth weight with P value of 0.047. This relation was statistically significant.

The relation between serum folate and birth weight was found to be statistically insignificant with P value of 0.59.

Table 4.1: Initiation of folate intake during pregnancy control group

<table>
<thead>
<tr>
<th>Item</th>
<th>Frequency</th>
<th>Percent</th>
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</thead>
<tbody>
<tr>
<td>in the first trimester</td>
<td>39</td>
<td>90.7</td>
</tr>
<tr>
<td>in the second trimester</td>
<td>3</td>
<td>7.0</td>
</tr>
<tr>
<td>in the third trimester</td>
<td>1</td>
<td>2.3</td>
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</table>
Table 4.2: Initiation of folate intake during pregnancy case group

<table>
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<tr>
<th>Item</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>first trimester</td>
<td>32</td>
<td>84.2</td>
</tr>
<tr>
<td>second trimester</td>
<td>5</td>
<td>13.2</td>
</tr>
<tr>
<td>Third trimester</td>
<td>1</td>
<td>2.6</td>
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Table 4.3: Intake of green leafy vegetables and meat control group

<table>
<thead>
<tr>
<th>Item</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>42</td>
<td>97.7</td>
</tr>
<tr>
<td>No</td>
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<td>2.3</td>
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</table>

Table 4.4: Intake of green leafy vegetables and meat case group

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<th>Item</th>
<th>Frequency</th>
<th>Percent</th>
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</thead>
<tbody>
<tr>
<td>Yes</td>
<td>32</td>
<td>78.0</td>
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<tr>
<td>No</td>
<td>9</td>
<td>22.0</td>
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Table 4.5: White blood cell count control group

<table>
<thead>
<tr>
<th>Item</th>
<th>Frequency</th>
<th>Percent</th>
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### Table 4.6: White blood cell count case group

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<thead>
<tr>
<th>Item</th>
<th>Frequency</th>
<th>Percent</th>
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<tbody>
<tr>
<td>&lt;10x10⁹</td>
<td>10</td>
<td>23.3</td>
</tr>
<tr>
<td>10-26x10⁹</td>
<td>33</td>
<td>76.7</td>
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### Table 4.7: Red blood cell count control group

<table>
<thead>
<tr>
<th>Item</th>
<th>Frequency</th>
<th>Percent</th>
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</thead>
<tbody>
<tr>
<td>&lt; 5x10⁶</td>
<td>42</td>
<td>97.7</td>
</tr>
<tr>
<td>5 – 7x10⁶</td>
<td>1</td>
<td>2.3</td>
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### Table 4.8: Red blood cell count case group

<table>
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<th>Item</th>
<th>Frequency</th>
<th>Percent</th>
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<td>&lt;5x10⁶</td>
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<td>90.7</td>
</tr>
<tr>
<td>5 – 7x10⁶</td>
<td>4</td>
<td>9.3</td>
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Table 4.9: Haemoglobin concentration control group

<table>
<thead>
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<th>Item</th>
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<th>Percent</th>
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</thead>
<tbody>
<tr>
<td>&lt;14 g/dl</td>
<td>5</td>
<td>11.6</td>
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<td>14-22 g/dl</td>
<td>38</td>
<td>88.4</td>
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Table 4.10: Haemoglobin concentration case group

<table>
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<th>Item</th>
<th>Frequency</th>
<th>Percent</th>
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</thead>
<tbody>
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<td>&lt;14 g/dl</td>
<td>15</td>
<td>34.9</td>
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<tr>
<td>14-22 g/dl</td>
<td>28</td>
<td>65.1</td>
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Table 4.11: Mean cell volume control group

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<th>Item</th>
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<td>&lt; 100 fl</td>
<td>17</td>
<td>39.5</td>
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<tr>
<td>100-120 fl</td>
<td>26</td>
<td>60.5</td>
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</tbody>
</table>

Table 4.12: Mean corpuscular volume case group

<table>
<thead>
<tr>
<th>Item</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;100 fl</td>
<td>12</td>
<td>27.9</td>
</tr>
<tr>
<td>100-120 fl</td>
<td>26</td>
<td>60.5</td>
</tr>
<tr>
<td>&gt;120 fl</td>
<td>5</td>
<td>11.6</td>
</tr>
</tbody>
</table>
Table 4.13: Platelet count control group

<table>
<thead>
<tr>
<th>Item</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;100</td>
<td>2</td>
<td>4.7</td>
</tr>
<tr>
<td>100-450</td>
<td>41</td>
<td>95.3</td>
</tr>
</tbody>
</table>

Table 4.14: Platelet count case group

<table>
<thead>
<tr>
<th>Item</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;100×10³</td>
<td>4</td>
<td>9.3</td>
</tr>
<tr>
<td>100−450×10³</td>
<td>39</td>
<td>90.7</td>
</tr>
</tbody>
</table>
Table 4.15 Mean, Minimum and Maximum Haematological value of the control group

<table>
<thead>
<tr>
<th>Items</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>TWBC</td>
<td>12.8791</td>
<td>4.34591</td>
<td>7.00</td>
<td>33.30</td>
</tr>
<tr>
<td>RBC</td>
<td>4.1221</td>
<td>0.46851</td>
<td>2.80</td>
<td>5.20</td>
</tr>
<tr>
<td>Haemoglobin Concentration</td>
<td>15.2744</td>
<td>1.69300</td>
<td>11.50</td>
<td>18.90</td>
</tr>
<tr>
<td>Packed Cell Volume</td>
<td>44.3288</td>
<td>4.33378</td>
<td>34.70</td>
<td>53.00</td>
</tr>
<tr>
<td>Mean Cell Volume</td>
<td>101.0558</td>
<td>4.53506</td>
<td>88.90</td>
<td>111.50</td>
</tr>
<tr>
<td>Mean Cell Haemoglobin</td>
<td>34.6116</td>
<td>1.85847</td>
<td>30.10</td>
<td>38.70</td>
</tr>
<tr>
<td>Mean Cell Haemoglobin Concentration</td>
<td>34.3116</td>
<td>0.91787</td>
<td>31.30</td>
<td>36.30</td>
</tr>
<tr>
<td>Platelets count</td>
<td>235.3721</td>
<td>76.44980</td>
<td>57.00</td>
<td>438.00</td>
</tr>
<tr>
<td>Reticulocytes</td>
<td>1.0209</td>
<td>0.59344</td>
<td>0.20</td>
<td>2.80</td>
</tr>
<tr>
<td>Serum folate</td>
<td>12.3859</td>
<td>3.18918</td>
<td>8.20</td>
<td>20.00</td>
</tr>
<tr>
<td>Red cell folate</td>
<td>1380.3651</td>
<td>69.73678</td>
<td>1302.00</td>
<td>1550.00</td>
</tr>
</tbody>
</table>
Table 4.16 Mean, Minimum and Maximum Haematological value of the case group

<table>
<thead>
<tr>
<th>Items</th>
<th>Mean</th>
<th>Std.Deviation</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>TWBC</td>
<td>12.0116</td>
<td>2.95459</td>
<td>7.00</td>
<td>18.40</td>
</tr>
<tr>
<td>RBC</td>
<td>4.3300</td>
<td>0.47085</td>
<td>3.30</td>
<td>5.30</td>
</tr>
<tr>
<td>Haemoglobin Concentration</td>
<td>14.6372</td>
<td>1.94863</td>
<td>11.00</td>
<td>17.6</td>
</tr>
<tr>
<td>Packed Cell Volume</td>
<td>43.9674</td>
<td>5.00931</td>
<td>33.60</td>
<td>55.50</td>
</tr>
<tr>
<td>Mean Cell Volume</td>
<td>102.7744</td>
<td>7.38403</td>
<td>84.50</td>
<td>120.10</td>
</tr>
<tr>
<td>Mean Cell Haemoglobin</td>
<td>34.0698</td>
<td>1.92793</td>
<td>27.60</td>
<td>37.40</td>
</tr>
<tr>
<td>Mean Cell Haemoglobin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration</td>
<td>33.8116</td>
<td>0.73199</td>
<td>32.50</td>
<td>35.70</td>
</tr>
<tr>
<td>Platelets count</td>
<td>247.8140</td>
<td>85.98983</td>
<td>48.00</td>
<td>436.00</td>
</tr>
<tr>
<td>Reticulocytes</td>
<td>0.9535</td>
<td>0.59815</td>
<td>0.20</td>
<td>2.6</td>
</tr>
<tr>
<td>Serum folate</td>
<td>10.6037</td>
<td>3.30492</td>
<td>4.4</td>
<td>18.01</td>
</tr>
<tr>
<td>Red cell folate</td>
<td>930.0428</td>
<td>226.93620</td>
<td>500</td>
<td>1345</td>
</tr>
</tbody>
</table>

Table 4.17 Red cell folate*birth weight cross tabulation case group:
<table>
<thead>
<tr>
<th>Red cell folate level</th>
<th>Birth weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>500-1013 ng/ml</td>
<td>1.5-1.9 kg</td>
</tr>
<tr>
<td>981 -1345 ng/ml</td>
<td>2 - 2.4 kg</td>
</tr>
</tbody>
</table>

P value 0.047

Table 4.18 Serum folate*birth weight cases group:

<table>
<thead>
<tr>
<th>Serum folate</th>
<th>birth weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.7-15.80 ng/ml</td>
<td>1.5-1.9 kg</td>
</tr>
<tr>
<td>4.4-18.01 ng/ml</td>
<td>2-2.4 kg</td>
</tr>
</tbody>
</table>

P value 0.59
Figure 4.1 Gender distribution control group
Figure 4.2 Gender distribution case group

Figure 4.3 Level of mother education control group
Figure 4.4 Level of mother education case group

Figure 4.5 Anaemia during current pregnancy control group
Figure 4.6 Anaemia during current pregnancy case group

Figure 4.7 Gestational age (control group).
Figure 4.8 Gestational age (case group).
Figure 4.9 RBCs folate among normal birth weight neonate

Figure 4.10 RBCs folate among low birth weight neonate
Discussion and Recommendations

5.1 Discussion

Birth weight is one of the most important pregnancy outcome parameters; it is strongly associated with infant mortality during the first year of life and influences later developmental processes as well (66). Folate required for growth reaches the maximal level in the last trimester (67), because of rapid growth of the fetus and the utero-placental system and fetal accumulation of folate stores.

According to Kramer, folate deficiency is potentially risk factor for intra uterine growth restriction (IUGR) in developing countries (68).

This case control study was conducted at Wad Medani Obstetrical and Gynaecological Teaching Hospital in the period from April to October 2016.

Eighty sex neonates (43 normal birth weight and 43 low birth weight neonates) were consecutively enrolled for the study.

The concentration of folate in umbilical vein blood probably reflects the concentration in the fetal blood shortly before delivery. The folate concentrations in serum and red blood cells in the samples from the neonate in this study therefore probably reflect the folate concentrations in the blood of the fetuses. Serum an red cell folate measured using electrochemiluminscence immunoassay.

In our study we found that the umbilical cord RBC folate status to be an important predictor of infant birth weight, with increasing cord RBC folate being associated with increasing infant birth weight. 13(30.2%) out of 43 low birth weight neonates were found to have low levels of cord red cell folate. These findings were in agreement with rondo et al and colleagues who compared the RBC folate in the cord blood of 315 growth restricted neonates and 321 normal
birth weight neonates and found that a greater percentage of low birth weight neonates had low red cell folate in cord blood (25.7%).

Hibbard et.al demonstrate clear association between folate level and birth weight with low levels among low birth weight neonates whose mothers did not receive folate during pregnancy\(^{(69)}\).

In our study, the folic acid intake during pregnancy appear to play an important role in determining birth weight with low birth weight being more common in those who were not received folate or irregularly received folate during pregnancy. Intake of green vegetables appear also to have minor association with birth weight with more low birth weight neonate among those who were not ate green vegetables.

Our study revealed that the level of mother education is an important factor in relation to birth weight. With prevalence of low birth weight neonate more among illiterate mothers.

Also we found that the regular antenatal care has an important role in having normal birth weight neonate with increase occurrence of neonatal low birth weight in those having no or irregular antenatal care.

CBC findings:

Cord blood is an ideal source for laboratory examinations in just-born neonates. It reveals not only fetal hematopoiesis but also the clinical conditions of those babies.

Haematological parameters were obtained using automated cell counter to measure

WBCs, RBCs, Hb, PCV, MCV, MCH, MCHC and platelets. The mean values for control group were RBC count 4.1221, Hb 15.2744, PCV 44.3288, MCV 101.0558, MCH 34.6116, MCHC 34.3116, TWBCs 12.4395 and platelets 235.3721.

The mean values for case group were RBC count 4.3300, Hb 14.6372, PCV 43.9674, MCV 102.7744, MCH 34.0698, MCHC 33.8116, TWBCs 12.4512 and platelets 247.8140.
Anaemia was more common among low birth weight neonate compared to control group (34.9% Vs 11.6%).

In those with low red blood folate levels:

High MCV was found in 5 cases and 8 cases were in the upper limit of normal.

Hypersegmented neutrophils was observed in 9 cases of those having low red blood cell folate levels.

This may be due to fact that the incidence of biochemical abnormalities due to folate deficiency is more fully documented than the incidence of haematological changes as in other conditions in which folate deficiency occurs the biochemical abnormalities are found far more frequently than overt megaloblastic anaemia since megaloblastic anaemia occurs only when the deficiency is extremely severe(70).

In control group:

5 cases had hypochromic microcytic anaemia most probably due to iron deficiency anaemia.

2 cases had platelet count less than 100 while their mothers had normal platelet count this most probably due to improper sample collection with clot formation.

Folate level:

Serum and red cell folate levels were measured by electrochemiluminscence immunoassay.

Statistically significant association was found between red blood cell folate levels and birth weight (P value of 0.047 in case group). Thirteen out of 43 in the case group (weight 1.5-1.7 Kg) had low red cell folate levels (500 ng/ml-625 ng/ml). This indicates the presence of relationship between folate levels in fetus and birth weight. Folate is required for DNA synthesis and cell division particularly during the third trimester when there is rapid proliferation of fetal cells which lead to depletion of tissue folate if there is no good supplementation with folate.

In those with low red blood folate levels:
5 cases out of thirteen who had low levels of red cell folate were preterm.

Prematurity is a leading cause of perinatal mortality and morbidity; therefore its association with folic acid supplementation is of major interest.

There are some hypotheses that could link reduced blood folate and prematurity. First, periconception folic acid supplementation may influence early placentation processes (71). In fact folic acid is potentially important in a number of crucial early stages of placental development, including extravillous trophoblast invasion, angiogenesis, and secretion of matrix metalloproteinases (72).

The role of folic acid in placentation could be supported also by the observation that increased homocysteine levels (on which folates have a lowering effect (73,74)) induce cytotoxic and oxidative stress on placental vascular and endothelial functions (75,76,77), and exposure of trophoblast cells to homocysteine may increase apoptosis (78). Periconceptional supplementation may therefore be beneficial in preventing pregnancy disorders associated with deficient placental development that could lead to preterm delivery (79,80,81,82,83).

No statistically significant association was found between the levels of serum folate and birth weight in the case group (P Value 0.59 in case group). This may be due to the fact that serum folate is a marker of recent dietary intake and it is subjected to prandial variation.
5.2 Conclusions

- Red blood cell folate is considered the most reliable biomarker of folate status, as it reflects tissue folate stores.
- A single measurement of serum/plasma folate reflects only the time of blood collection and cannot differentiate between occasional low dietary intake of the vitamin and folate deficiency.

- Our study revealed that there is clear association between folate deficiency and increase incidence of low birth weight and preterm delivery.
5.3 Recommendations:

- Encouragement of folate supplementation during pregnancy which will not only reduce the risk of neural tube defect but also reduce the risk of low birth weight which have a long term morbidity and mortality.

- Educational programs to improve the awareness about the importance of folate supplementation during pregnancy are highly recommended.

- Further community based studies should be carried out to measure the magnitude of folate deficiency in other parts of Sudan.
References


57- de Benoist B. Conclusions of a WHO Technical Consultation on folate and vitamin B12 deficiencies. Food and Nutrition Bulletin 2008; S238-244.


77. van Mil NH, Oosterbaan AM, Steegers-Theunissen RPM. Teratogenicity and underlying mechanisms of homocysteine in animal models: a review. Reproductive Toxicology. 2010;30(4):520–531.


Appendix 1

University of Gezira
Faculty of medicine
Department of Pathology-Haematology
MD clinical pathology
Questionnaire
Serum and red cell folate Levels among low birth weight neonates in Wad madani Obstetrical and Gynaecological Teaching Hospital from April 2016 To October 2016

Date : / / 2016 Patient number

Personal Data:

Name : .................................................................

Age : [ ] hrs

Tribe :
Residence :……………………………………………………………………

Level of mother education : Illeteate ☐ Read/write ☐
   Secondary ☐ University ☐

Mother occupation :……………………………………………………………………

Contact number: ………………………………………………………………………

The number of siblings?...............

Folic acid intake during the pregnancy period?
   ☐yes   ☐ No

If yes When she started intake?

In the first trimester ☐ in the second trimester ☐ in the third trimester ☐

Anaemia during pregnancy?
   Yes ☐ No ☐

Other pregnancy comorbidity?

DM ☐ HTN ☐ placental abruption ☐ preeclampsia ☐ malaria ☐

NO ☐ ☐

Past history of low birth weight neonate?

Yes ☐ No ☐

Past history of abortion or stillbirth?

Yes ☐ No ☐
History of neural tube defect?
Yes ☐ No ☐

History of preterm delivery?
Yes ☐ No ☐

History of twins?
Yes ☐ No ☐

History of blood transfusion?
Yes ☐ No ☐

History of bleeding?
Yes ☐ No ☐

Family history of low birth weight neonate?
Yes ☐ No ☐

High moderate low

Drugs during pregnancy?
Yes ☐ No ☐

If yes mention drugs?

..............................

regular antenatal care?
Yes ☐ No ☐

intake of green leafy vegetables and meat?
Yes ☐ No ☐

Alcohol intake?
Yes ☐ No ☐
Last menstrual period:

……………………………

Gestational age:

Term ☐ preterm ☐

Mode of delivery:

Normal vaginal ☐ cessearean section ☐ forceps assisted ☐ vaccum assisted ☐

The child birth weight:

……………………….Kg.

Examining the neonate for:

Cephalohaematoma ☐ coarse feature ☐ congenital malformation ☐

CBC result:

Counts: ...................................................................................................................
...................................................................................................................
...................................................................................................................
...................................................................................................................
...................................................................................................................

Prepheral blood film findings:

....................................................................................................................
Serum folate level:.............................

Red cell folate level:.........................

Appendix 2

Plates
Plate (1): umbilical cord sampling
Plate (2): Umbilical cord serum sample
Plate (3): umbilical cord blood samples
Plate (4): Red blood cell and serum folate reagents
Plate (5): Cobas e 411 analyzer

Plate (6): sample testing
Appendix 3

percentile chart