FOLIC ACID DEFICIENY AND PREGNANCY COMPLICATIONS

(A study of 887 women)

by

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Folic acid deficiency is an important contributory factor in the etiology of various pregnancy complications. Its exact role in each of these conditions may be difficult to define, but the high incidence of certain pregnancy complications in association with folic acid deficiency is shown by various workers.

Metabolic Functions of Folic Acid: Folic acid or pteroylglutamic acid, a water soluble vitamin, gets converted into folinic acid in the body and plays various important roles (Goodman & Gilman, 1966), viz:—

(1) Purine synthesis [via formylation of glycinamide ribonucleotide (GAR) and 5-amino-4-imidazole carboxamine ribonucleotide (AICAR)].

(2) Biosynthesis of pyrimidine nucleotide (via the methylation of deoxyuridylic acid to thymidylic acid).

(3) Three amino-acid conversions:

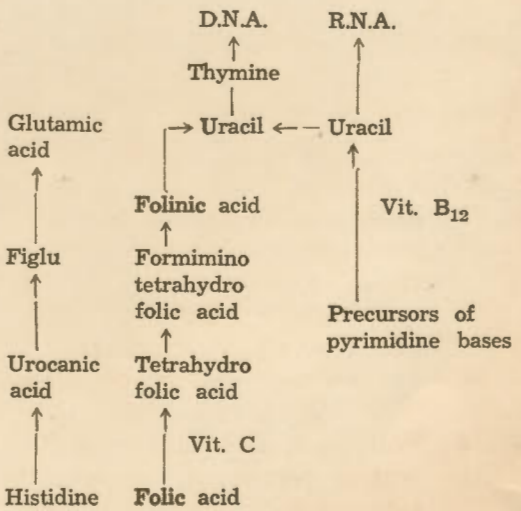
(a) Inter-conversion of serine and glycine.

(b) Catabolism of histidine to glutamic acid.

(c) Conversion of homocysteine to methionine.

(4) The generation of formate into the so-called formate pool and the utilization of formate there from.

The role of folic acid in nucleic acid metabolism can be easily understood from the following chart:



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Thus, folic acid is required for every growing cell and as during pregnancy, foetal cells divide with tremendous speed, the need for it during pregnancy is great. Maternal tissues like uterus, blood cells and blood vessels also expand by cell division, thus requiring additional folic acid. Chanarin *et al* (1967) have shown that deficiency of folic acid may lead to defective or retarded growth of foetus, placenta or both. This retarded or defective growth may be due to delayed placental maturity leading to multiple micro-infarcts and avascular villositis resulting from nutritional deficiency, as shown by Bazso (1966).

Natural sources: Folic acid occurs naturally in green and leafy vegetables, yeast and liver.

Daily requirement:

The minimal daily requirement for non-pregnant normal women is 50 ugm. During pregnancy the requirement increases manifold and varies between 3 mg. per day to 5 mg. per day, as shown by Spies (1949).

Measurement of Folate Activity:

The state of folate activity of the body can be estimated by various ways:

(1) Figlu test
(2) Folic acid clearance test
(3) Serum folic acid levels (microbiological assay)
  (a) Using L. Casei
  (b) Using L. citrovorum
  (c) Using Streptococcus faecalis
(4) Folic acid contents in R.B.C. which represent tissue contents of folic acid.

Figlu test:

The principle used in this method is the arrest of histidine catabolism in the absence of folic acid so that glutamic acid is not formed.

Ten gm. of histidine are given orally and urine is collected at that time and after 5 hours, when the maximum excretion takes place. Samples are examined electrophoretically for Figlu. Normal rate is 15 mug./ml. of urine.

Opinions regarding efficacy of this test are divided. Hibbard (1962) found it 100% reliable, Chanarin *et al* (1963) 50%, while Husain *et al* (1963) did not find it at all dependable.

The objections to Figlu test are:

(1) Oral histidine is slowly absorbed.

(2) Renal threshold for histidine is lowered in pregnancy.

(3) Part of the histidine may be utilized by the foetus itself for protein synthesis.

Folic acid clearance test:

This test comprises of intravenous administration of folic acid (15 mcg./kg. weight) and studying the rate at which blood is cleared off the folic acid due to tissue uptake of the same. The greater the demand by the tissues, the faster will be the clearance rate. Taking normal non-pregnant clearance rate as zero per cent (or standard) the results at various periods of gestation, etc., are as follows:

Non-pregnant	..	0%
12 weeks gestation	..	8%
20-24 weeks gestation	..	33%
36-40 weeks gestation	..	68%
Twin pregnancy	..	100%
Megaloblastic anaemia	..	100%

Thus, the demand for folic acid, which is manifested by rapid clearance from blood, increases from the 12th week to 40th week of pregnancy, and in twin pregnancy and megaloblastic anaemia, the demand is so great that the total amount of extraneously administered folic acid is taken up by the tissues.

Serum Folic Acid Estimations:

Serum folic acid levels are estimated by microbiological assay method using various organisms, e.g.

(1) L. casei, which estimates conjugated and free folic acid, as well as folinic acid,

(2) L. citrovorum which estimates folinic acid only,

and, (3) Strepto. faecalis estimating free folic acid and folinic acid.

Normal serum folic acid levels:

Using microbiological assay methods, various workers found normal serum folic acid levels as follows:

Cooper and Lowenstein (1961)	More than 3	mug./ml.
Herbert, et al (1960)	7.5-24	„ „
Waters and Molin (1961)	5.9-21	„ „
Davis and Kelley (1962)	More than 1.8	„ „

Serum folic acid levels normally also vary in non-pregnant, early pregnant and late pregnant states. This is seen in Table I.

Material and Method

The present series include 887 cases of serum folic acid estimations done by Herbert's (1960) microbiological method. Proteins are first precipitated and serum is then incubated with L. casei inoculum. Turbidity obtained is then compared with standard graphs and results are calculated in mug./ml. of blood. Distribution of cases is shown in Table II.

TABLE II

Folic acid estimations in different clinical groups

	Number of cases
Anaemia	75
Toxaemia	45
Accidental haemorrhage	11
Still births	70
Abortions	69
Bad obstetric history	210
Premature delivery	25
Twins	30
Congenital foetal malformation	12
Treated with prophylactic folic acid therapy	245
Control or normal — Non-pregnant	25
— Pregnant	50
— Post-partum	20
Total	887

Normal cases: The normal value varied from 3.5 to 15 mug./ml., with mean of 7.5 mug./ml., in non-pregnant state and having mean value of 6.2 mug./ml. during normal pregnancy.

TABLE I

Serum folic acid levels in non-pregnant and pregnant women

	Krishna Menon (1965)	Solomons et al (1962)
Non-pregnant	18.8 mug./ml.	7.8 mug./ml.
Early pregnancy	8.8 „ „	5.8 „ „
Late pregnancy	12.67 „ „	4.61 „ „
Post-partum	—	4.0 „ „

In our study the mean folic acid levels were lower in young primipara and elderly grand multipara as compared to patients falling in the age group 25-30 years and those having 2nd or 3rd pregnancy. The lower levels in young primipara are explained by the fact that their tissues still require folic acid for their own growth and in elderly grand multipara the levels may be low because of continuous deprivation of folic acid due to repeated pregnancies. The levels were also higher in second trimester of pregnancy as compared to first and third trimesters; the significance of this cannot be explained clearly.

Anaemia:

Folic acid deficiency plays an important role in the genesis of anaemia of pregnancy. Though iron deficiency is of great importance, multiple deficiency is more common, specially in our country.

Table III shows the incidence of various types of deficiency anaemias.

TABLE III

Incidence of various types of anaemias

	Benjamin et al (1966)	Krishna Menon (1965)	Dass et al (1967)
Pure iron deficiency	15.4%	35%	52 %
Deficiency of iron and folic acid	39.2%	60%	39.6%
Deficiency of iron, folic acid and vitamin B_{12}	15.0%	—	—

The rest of the cases in these studies were of rare types.

In the present series, 17 out of 75 anaemic cases had folic acid deficiency indicated by low serum folic acid levels (less than 3 mug./ml.). As bone marrow was not examined in all of them, the incidence of megaloblastic erythropoiesis cannot be judged.

This high incidence of associated folic acid deficiency had led many workers to start routine folic acid as a prophylaxis in all the cases of anaemias of pregnancy.

During comparative studies various interesting and important observations were noted by people working in this field.

(1) Administration of parenteral iron resulted in unmasking of folic acid deficiency symptoms in 20% of Bonnar's (1965) cases. This may be probably of the increased utilization of haemopoietic factors following the intense stimulation of erythropoiesis due to rapid replenishment of iron stores in deficient cases.

(2) While studying 81 anaemic women, it was observed by Metz *et al* (1967) that the mean rise of haemoglobin was higher when additional folic acid was given, than in cases treated with pure iron therapy (4.15 gm./1000 ml. in latter as compared to 4.73 gm./100 ml. in former cases). Besides, the cases which failed to respond to iron, quickly improved when folic acid was added later on.

(3) Iron deficiency can actually result in biochemical and morphological changes of folic acid deficiency because an enzyme concerned in folic acid metabolism, namely glutamate

formimino transferase, is iron dependent.

(4) Megaloblastic anaemia is of more severe variety than pure iron deficiency type and occurs later in pregnancy. It is often diagnosed only when some patients fail to respond to iron and administration of folic acid dramatically improves them.

Timely administration of proper dosage of folic acid can entirely prevent the development of megaloblastic anaemia in most of the cases (Lowenstein *et al* 1955).

A group of 235 cases having anaemia of pregnancy with haemoglobin between 5-10 gm.% were treated with iron and folic acid parenterally (Uniferron-F injections). Of these, 164 had full term babies, 4 had premature deliveries, 1 had abortion, 38 are continuing pregnancy, 19 are lost to follow-up and 9 discontinued the therapy. Thus, of 169 cases, whose outcomes are known, 164 had full term deliveries and 5 had foetal loss, the foetal salvage being 97%.

Various studies carried out by Willoughby (1967) has showed that 300 ugm./day of folic acid is the optimum prophylactic dose which would prevent megaloblastic erythropoiesis if given from early pregnancy, and has no danger of precipitating the neurological symptoms in cases of unsuspected associated B_{12} deficiency. Besides, a loading dose of B_{12} can always be given at the beginning of therapy to obviate any possibility of such a danger.

Toxaemia

Folic acid deficiency has been said to play a role in the etiology of toxaemia of pregnancy. Though its exact role is not understood, nutritional deficiency might lead to microinfarcts and other changes in the placenta thus altering its function. (Bazso, 1966).

The incidence of folic acid deficiency was 33% in 45 cases of toxaemia in the present study. There was no correlation between the severity of toxaemia and the extent of folic acid deficiency. But when the severity of toxaemia was the same in the two groups, foetal salvage was better with normal folic acid level as compared to folic acid deficient group. This is shown in Table IV.

It is obvious from this table that amongst toxaemic cases, foetal salvage was 7 out of 15 cases with folic acid deficiency, while in 19 out of 25 cases the foetus survived when folic acid levels were normal.

TABLE IV

Analysis of foetal salvage according to grades of toxaemia

	Folic acid deficient group		Normal cases	
	Salvage	Loss	Salvage	Loss
Mild	3 cases	2 cases	6 cases	0 case
Moderate	3 cases	4 cases	7 cases	5 cases
Severe	0 case	2 cases	3 cases	1 case
Eclampsia	1 case	0 case	3 cases	0 case
	15 cases		25 cases	

Premature Delivery

The incidence of premature delivery is considerably higher in our country as compared to Western countries. This may be probably because of poor nutritional status and other factors prevalent in our country. It is considerably high in megaloblastic anaemia as compared to pure iron deficiency type. This is because megaloblastic anaemia is usually of severe variety and occurs in later months of pregnancy. Hibbard (1964) found the incidence of premature delivery to be more common in such cases. Incidence of folic acid deficiency in patients who had recently given birth to premature babies was found to be 20% in the present study.

Twin Pregnancy

The association of folic acid deficiency and twin pregnancy is observed by many workers in their studies. The high incidence of folic acid deficiency in twin pregnancy is shown by them.

TABLE V

Incidence of folic acid deficiency in twin pregnancy

Author	Incidence of folic acid deficiency
Hibbard (1964) ..	27%
Lillie (1962) ..	17%
Mackenzie and Abbot (1960) ..	24%
Lowenstein et al (1965) ..	26%
Present study ..	20%

Coyele and Geohegan (1962) studied 6017 pregnant cases. Megaloblastic anaemia was found to be three times commoner in twin pregnancy than otherwise. Chanarin (1967) found it to be eight times commoner.

Prophylactic folic acid therapy is very important in twin pregnancy and proper dosage can prevent folic acid deficiency in all the cases. Lillie completely prevented megaloblastic anaemia in 41 cases of twins by routine administration of 5 mg. of folic acid. In the second group of 42 cases with twin pregnancy that did not receive any folic acid, megaloblastic anaemia accounted for 7 out of 42 cases, i.e. in 16.6% of cases.

Abortions

Nelson (Ciba Foundation Symposium) showed experimentally that if folic acid deficiency was produced in rats, deaths, reabsorption of foetus or conditions similar to "blighted ovum" resulted, depending upon the period of gestation. Administration of folic acid antagonist in human beings resulted in abortions in 9 out of 10 cases and the remaining one had congenital malformation incompatible with life. Martin (1964 and 1965) compared 160 normal pregnant patients with 19 cases of threatened abortions and 35 cases of inevitable abortions. He found 98% correlation between threatened abortion and low folic acid level and 95% correlation between inevitable abortion and high or normal folic acid levels. In the present study, 30% of threatened abortion cases had deficiency and 90% of inevitable complete abortions had normal to high levels. Martin (1964 and 1965) feels that high levels in inevitable incomplete or complete abortions are caused by (1) slackening of demand of folic acid on the part of the foetus due to death, or (2) reabsorption of folic acid from the dead foetus and placenta. The low incidence (only 30%)

of folic acid deficiency in our series of threatened abortion cases may be probably because many of these ova might have been dead or dying, though clinically the actual process of expulsion of the ovum had not started and hence they were clinically labelled as cases of "threatened" abortion.

Martin (1965) had shown that folic acid deficiency when corrected in the pre-conceptional or early conceptional stage can prevent an abortion, but it is of no value after the onset of bleeding. Herein lies the value of detecting folic acid deficiency in post-abortal cases and cases of recurrent abortions and treating them in the non-pregnant stage before they conceive again or during early pregnancy before the onset of bleeding.

Congenital foetal malformations

One of the etiological factors of congenital malformation is deficiency of folic acid. The pathology underlying congenital foetal malformation is the same as that of abortion but acting at a different stage of gestation or in insufficient severity to lead to foetal death (Hibbard 1964). Not all types of malformations are related to folic acid deficiency and these conditions are not so very frequent as to obtain statistically significant data. In the present study the results are as follows:

TABLE VI

Foetal malformations associated with low serum folic acid levels

	Cases	Low level
Anencephaly	6	3
Hydrocephalus	6	0

In mothers of anencephalic babies, 3 out of 6 cases had less than 3 mug./ml. and the remaining 3 had levels between 4-6 mug./ml. None of the cases had levels more than 6 mug./ml.

Stillbirths

Seventy women delivering full term stillborn babies without any clinically demonstrable pathology were studied for folic acid levels after delivery. Seventeen out of 58 cases showed low levels giving an incidence of 29% deficiency. Six cases revealed normal levels after 6-8 weeks without any therapy, while the remaining cases showed persistent low levels and required folic acid therapy later on.

Accidental haemorrhage

The role of folic acid deficiency in causing accidental haemorrhage is the most controversial aspect of this problem, as can be seen from Table VII.

TABLE VII

Incidence of folic acid deficiency in accidental haemorrhage

Author	Incidence of deficiency
Coyele and Geoghegan (1962)	45%
Hibbard and Jeffcoate (1966)	97%
Krishna Menon (1966)	No correlation
Present study	27.2%

Hibbard (1964) suggests that the basic cause of abruptio placentae is either a chronic dietary deficiency exacerbated by repeated and frequent child-bearing or an error in metabolism of folate not demonstrable before pregnancy but becoming evident within a few weeks of conception. Abruptio placentae can be

prevented if folic acid deficiency is corrected before conception or in the very early conceptional stages. It is of no value if given in mid-pregnancy, once the Figlu test becomes positive, and even if the Figlu test then becomes negative by folic acid therapy, abruptio placentae cannot be prevented. This chronic deficiency explains the recurrence of abruptio placentae and the fact that it is common in multipara. Abruptio placentae occurs in 14% of cases of megaloblastic anaemia as compared to 2.5% found in normal cases.

Prophylactic folic acid therapy

The value of prophylactic folic acid therapy during pregnancy has to be judged from three points of view: 1. Cost. 2. Dangers. 3. Advantages.

Cost: Its proved role in the prevention of megaloblastic anaemia leaves no doubt for considering the cost of routine folic acid therapy, which is anyhow not costly.

Dangers: The danger of precipitating neurological symptoms by folic acid therapy in cases with co-existing B_{12} deficiency was perhaps overemphasised in the past. Pernicious anaemia is so rare in young pregnant women that withholding the therapy because of this danger is not justifiable. Again, the dose in which routine prophylaxis is carried out is not large enough to cause any such adverse effects and a loading dose of B_{12} at the beginning of the therapy will obviate even the slightest danger.

Advantages: Advantages of prophylactic folic acid therapy during pregnancy are now well understood:

(1) It is definitely proved to prevent development of megaloblastic anaemia if given in proper dosage and time. This is shown by Lowenstein *et al* (1955), Willoughby (1967) and many others.

(2) It can reduce the incidence of abortions, abruptio placentae, etc., if given in the preconceptional or early conceptional states.

(3) Prophylactic therapy with folic acid in cases with repeated foetal loss where no other cause can be detected was found to be very beneficial as regards foetal salvage.

All the cases in our "Bad Obstetric History" (B.O.H.) O.P.D. were given routine folic acid, 1 mg. tablets, and the foetal salvage was studied. In those cases where no other cause was present, folic acid therapy along with general line of treatment improved the foetal salvage considerably.

Folic acid levels in these B.O.H. cases varied from 0.7 to 20 mug./ml. and 20% of them were found to be deficient.

In a group of 245 cases treated prophylactically with folic acid who had no other abnormality present at that time, folic acid levels ranged from 4 to 20 mug./ml., with a mean of 7.8 mug./ml. None of the cases had folic acid levels of less than 3 mug./ml. and only 10 cases had levels less than 6 mug./ml. Foetal salvage was 88%.

Summary

(1) There is a definite place for routine folic acid therapy during pregnancy to prevent pregnancy complications like abortion, abruptio placentae etc. and megaloblastic anaemia.

(2) In the toxaemic group foetal salvage was obtained in 19 out of 25 toxaemic patients with normal folic

acid levels, while only 7 out of 15 survived in the deficient group.

(3) There is an association between twin pregnancy and folic acid deficiency.

(4) Prophylactic folic acid along with general care of the patient improves foetal salvage in cases with repeated foetal loss where other etiological factors are ruled out or treated. Foetal salvage in the present study of 210 such cases was 88%.

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