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Original Research Article

Does folic acid reduce congenital anomalies and adverse pregnancy outcomes in women received long-term low dose methotrexate?

Ahmed M. Farag*, Safaa A. Ibrahim

Department of Obstetrics and Gynecology, Faculty of Medicine Zagazig University, Zagazig, Egypt

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***Correspondence:**

Dr. Ahmed M. Farag,

E-mail: ahmed.farag@zu.edu.eg

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ABSTRACT

Background: Methotrexate (MTX) which is folic acid antagonist is used to treat many diseases, including rheumatic, inflammatory, autoimmune disorders or malignancies; it is also used for the medical treatment of ectopic pregnancy and termination of pregnancy. The objective of this study was to assess the impact of folic acid in preventing congenital anomalies (CAs) in the fetus of pregnant that take long-term low dose methotrexate.

Methods: The study included 1569 with history of low dose methotrexate therapy <25 mg/week that stopped one month before pregnancy, they were divided into 3 groups; Group 1 comprised 272 pregnant women, Group 2 comprised 367 pregnant women both groups take folic acid 4-5 weeks preconception and for the first 12-13 weeks gestation, the dose in the 1st group was 0.4 mg/day while in 2nd Group 4 mg/day, Group 3 comprised of 930 pregnant disease-matched with no folic acid. Group 4 comprised of 1015 pregnant no underlying autoimmune and no folic acid intake.

Results: The overall rate of CAs and cardiovascular CAs were higher in fetuses of Group 3 (OR: 1.7; 95% CI: 1.1-2.7) and (OR: 2.7; 95% CI: 1.4-5.2) respectively compared to the overall rate of CAs and cardiovascular CAs in other groups. Other adverse outcomes as abortion, preterm delivery, preeclampsia, placenta abruption, PROM, low birth weight, admission to NICU, IUGR and IUFD were higher in Group 3 and 4 than in Group 1 and 2. There were no significant differences in umbilical arterial PH. The Apgar scores <7 at 1, 5 min and gestational age at delivery were higher in Group 1 and 2 than Group 3 and 4.

Conclusions: There was a certain reduction in methotrexate teratogenic effect on the fetuses with folic acid intake during pregnancy, these pregnancies might benefit from taking of folic acid in high doses before conception and during the first trimester of pregnancy and improvement in fetal and maternal outcomes but the explanation of this effect requires further study.

Keywords: Congenital abnormality, Fetal outcomes, Folic acid, Maternal, Methotrexate, Pregnancy

INTRODUCTION

Methotrexate (MTX) which is folic acid antagonist is used to treat many diseases, including rheumatic, inflammatory, autoimmune disorders or malignancies; it is also used for the medical treatment of ectopic pregnancy and termination of pregnancy. Dose varies according to indication of treatment, ranging from 2.5 mg/week to 30 mg/week (for the treatment of rheumatoid

arthritis), 1 gm/m² of body surface area (for the treatment of some types of malignancy). MTX was found to be teratogenic in humans with high doses.¹⁻⁴

Aminopterin (folate antagonist) and MTX has been associated with certain fetal anomalies: CNS anomalies, like anencephaly, spina bifida, hydrocephalus, and mental retardation; skeletal deformities, like absent or partial bones ossification, lambdoid sutures synostosis,

micrognathia, cleft lip or palate, depressed nasal bone, hypertelorism, limb shortage, syndactyly, absent digits; and dextrocardia and IUGR.^{5,6}

Folate deficiency may be due to insufficient dietary intake, defect in utilization, alcohol abuse, malabsorption, hepatic diseases, increased requirements in pregnancy and lactation, smoking, deficiency of enzymes responsible for FA formation or interference by drugs like anti-convulsant, methotrexate, oral contraceptives, sulfasalazine, trimethoprim and pyrimethamine.⁷⁻¹¹

The functional folate insufficiency (one of the commonest nutritional deficiencies) is emerging in participating in maternal adverse outcomes as pre-eclampsia, which is a main cause of maternal mortality (15-20% in developed countries) and morbidities (acute and long-term), perinatal deaths, PTL and IUGR.^{12,13} Despite the improvement of antenatal care, the scope of pre-eclampsia has not decreased. Several studies alluded that women who develop preeclampsia had increased peril of cardiovascular complications later on during life, in particular, hypertension, ischemic heart disease, venous thromboembolism, stroke, and even death.¹⁴⁻¹⁷ Primary prevention of these adverse pregnancy outcomes is a fateful project priority, including pre-conceptional approaches.¹⁸ Furthermore, finding inexpensive and simple ways to prevent these maternal and neonatal adverse outcomes is eminent scope.¹⁹⁻²¹

The target of this work to study the effect of folic acid supplement in higher dose (4 mg/day) before conception and during first trimester of pregnancy in comparison with the commonly recommended dose (0.4 mg/day) on reducing the peril of all CAs and improving pregnancy outcomes in women received long-term low dose Methotrexate.

METHODS

This was a prospective observational study of women who were under low dose methotrexate (< 30 mg/week) for a long time for treating autoimmune diseases and was planning for pregnancy, they were advised for stopping the drug for 1 month before pregnancy. Cases recruited from antenatal clinic (ANC) and/or maternity ward at Zagazig University Hospitals (ZUH), Zagazig, Egypt over the period from June 2015 to 28th June 2018. The study sample included 639 pregnant women subjected to pre-conceptional MTX (367 take-ups 4 mg/day, 272 take-ups 0.4 mg/day 4-5-week pre-conception till 12-13-week gestation), 918 disease-matched comparison women without methotrexate, and 1015 comparison women without autoimmune diseases.

Study protocol

The study protocol was approved by ZUH ethical committee. Participating cases were counseled, the intervention was clearly explained, and an informed

written consent was then taken from all of them. During booking (first visit), data recorded include age, parity, maternal weight and height, ethnicity, social condition (career of the mother and her partner), consanguinity and index of multiple deprivations. Medical history, obstetric history, and past history were taken. FA intake (onset and dose of supplementation), and time of first visit in pregnancy were collected plus additional information relating to pregnancy complications (gestational diabetes, antepartum hemorrhage, pregnancy-induced hypertension, and pre-eclampsia). Data of neonates included gestational age (from booking scans) and birth weight. GE Voluson E6, curvilinear probe 3.5 MHZ was used during the survey by a single sonographer.

At the second visit, compliance with vitamin intake was checked and the women had a urine pregnancy test, vitamin tablets were supplied for the next three months, and were inquired to get pregnant. If a woman did not conceive within the first three months, another three-month supply of vitamin tablet was advised. If a woman did not conceive, she was excluded from the study.

The purpose of the third visit was confirmation of pregnancy by a urine pregnancy test and ultrasonography immediately after the first missed menstrual period. Compliance with vitamin intake was also checked and vitamin tablets were supplied.

At the fourth visit, compliance with vitamin intake was again evaluated and supplemented pregnant women who were 14 weeks' gestation (calculated from the first day of the LMP) were finally enrolled in the study and referred to a regional antenatal care clinic (ACC).

Inclusion criteria

- Pregnant women that take methotrexate not more than 30 mg/day that stopped one month before the current pregnancy maternal age 20-35 years old, singleton fetus was eligible and approached for recruitment no smoking or consumption of alcohol.

Exclusion criteria

- Women with the following conditions were ineligible and excluded: maternal age <20 or >35 years old, multiple pregnancies, obesity (BMI ≥ 25 Kg/m²), diabetic or hypertensive patients.

Random assignment of women to receive 0.4 mg of FA (referent cases) or 4 mg of FA (treatment cases) daily. During ANC visit enrolment, general and abdominal examinations, past history and obstetric history were carried out, the fetal heart rate was checked, and findings were recorded. Routinely needed investigations, including CBC and urine analysis, liver and kidney function tests were arranged. In cases with prior regular menses, GA was calculated from the date of LMP. Otherwise, GA was estimated from early ultrasound scan

(USS) at 7 weeks gestation and was verified by the 13th week USS.

Ultrasound anomaly scan was accomplished at 20-24-week gestation. If any anomaly was detected, women were counselled to continue the pregnancy or terminate it. Women were then followed up by the routine antenatal clinic protocol, re-examined every month until 28 weeks gestation and every 2 weeks until 36 weeks gestation then every week until delivery. Those who developed any adverse pregnancy outcome were seen more often or admitted accordingly to the maternity ward.

Statistical analysis

Was done using SAS version 8.02 (SAS Institute, Cary, NC, USA) for statistical analysis of data. First, the major maternal variables in the control and case groups were evaluated using a t-test for quantitative while C2 was used test for categorical variables in comparing cases with and without maternal autoimmune diseases as a reference.

The preponderance of other maternal diseases, drug supplementation particularly folic acid and multivitamins containing folic acid during pregnancy, in addition to pregnancy complications were compared between the control and case groups using odds ratios (OR) with 95% confidence intervals (CI). We compared the appearance of specific CA in the case group and in all matched controls and adjusted OR with 95% CI were assessed in conditional logistic regression models. The distribution of specified CA-groups was evaluated in the children of pregnant women with a history of methotrexate therapy according to the dosage of folic acid supplement 4 mg/day versus 0.4 mg/day from 4-5 weeks before pregnancy till the end of the 1st trimester during the survey of pregnancy outcomes.

Cases with CAs detected by ultrasound were divided into a single (affection of one organ only) and multiple (affecting two different organ systems at least and concurrent two or more CAs in the same case). The total prevalence of cases (birth + fetal) with CA diagnosed from the second trimester of pregnancy. Controls were the newborn infants without CA.

Well-defined four CAs groups of informative offspring

- Malformed fetuses diagnosed antenatally and terminated in the second, and very rarely in the third, trimester;
- Stillborn fetuses (late fetal death after the 28th week of gestation); and
- Live-born infants. Each member of twins and triplets was evaluated separately. Isolated and multiple CAs were differentiated. Heart murmur without cardiological confirmation, deformations, minor anomalies, and genetic diseases were excluded.

Three-time windows were used for the detection of CAs in both cohorts

- Antenatally diagnosed fetal abnormalities in terminated pregnancies followed by a pathological description. There are four recommended ultrasound examinations during pregnancy and the number of ultrasound examinations ranged from 4 to 12 in our study. All available medical records (ultrasound films, fetal pathological description, etc.) were collected to render a rigorous diagnosis of fetal defects.
- At birth, all deliveries of newborn infants and stillborn fetuses occurred in inpatient clinics of obstetrics and the attendants of labor were obstetricians during the period of the study. Most neonates were also examined by pediatricians in inpatient clinics of obstetrics. In addition, malformed infants were cared for in outpatient and inpatient pediatric clinics. An autopsy was compulsory in infant death, and it was requested in stillborn fetuses. If a completed pregnancy outcome certificate indicated any CA, all available medical records (detailed physician's description, autopsy record, etc.) were obtained to evaluate clinical and pathological information as much as possible.
- At about the 12th month after birth, all infants were called for personal examination and were checked by one pediatrician "blindly." If families did not participate in this examination, we repeated the invitation. If there was still no response, we contacted the pediatrician of the infant and obtained the case history, particularly data regarding CAs diagnosed after birth, a recent death, and serious and/or chronic disorders. Thus, all live-born infants except cases of postnatal death had a double check (birth and follow-up data regarding CA). Autopsy reports were available on all stillborn fetuses and infant demise.

RESULTS

Demographic data of pregnant women were similar in general (Table 1), but usage of systemic steroid or DMARD (drug modulating activity of rheumatoid arthritis) was lower in the women without underlying autoimmune disorder, also history of preterm birth or abortions were lower in the same group, this may be due to underlying congenital anomalies in other groups. The proportion of diabetes mellitus, epilepsy, and CAs were 0.5 versus 0.2%, 0.5 versus 0.1%, and 2.2 versus 0.3%, respectively, in the mothers of previous low doses of methotrexate therapy and other with no underlying autoimmune diseases.

The different fetal anomalies in cases with underlying autoimmune disorder and received folic acid (either 0.4 or 4 mg/day) compared to those without folic acid supplement and pregnant women with no underlying autoimmune diseases were presented in Table 2, 3.

Table 1: Comparison between maternal characteristics in the studied groups.

	Folic acid intake					No folic acid				
	0.4 mg/day (N = 272)	4 mg/day (N = 367)	OR	(95% CI)	p-value	Disease-matched group (N = 930)	Pregnant cases with no underlying autoimmune disease (N=1015)	OR	(95% CI)	P-value
Maternal age at delivery (y)	34.0±3.9	33.4±4.6			0.578	34.0±5.0	33.3±4.3			0.731
Primigravida	82 (30.1%)	112 (30.5%)	1.06	0.36-3.14	0.866	270 (29.03%)	300 (29.5%)	1.22	0.29-5.17	0.917
Multipara	190 (69.9%)	235 (69.5%)	0.64	0.22-1.85	0.569	660 (70.97%)	715 (70.5%)	0.54	0.13-2.22	0.595
Previous children with anomalies	32 (11.7%)	42 (11.4%)	3.67	1.06-12.74	0.074	108 (11.6%)	10 (0.9%)	1.69	0.18-16.11	< 0.001
Socio-economic class										
High	99 (36.4%)	111 (30.2%)				312 (30.7%)	310 (30.5%)			
Middle	89 (32.7)	115 (31.3%)	0.37	0.04-3.25	0.619	401 (43.11%)	415 (40.8%)	1.69	(0.18-16.11)	0.83
Low	84 (30.9)	141 (38.4%)				217 (23.3%)	290 (28.5%)			
Systemic DMARD use	190 (69.9%)	235 (69.5%)	0.64	(0.22-1.85)	0.569	660 (70.97%)	0 (0%)	0.54	(0.13-2.22)	< 0.001
Systemic steroid use	82 (30.1%)	112 (30.5%)	1.06	(0.36-3.14)	0.866	270 (29.03%)	15 (1.4%)	1.22	(0.29-5.17)	< 0.001
Prior preterm delivery	32 (11.7%)	42 (11.4%)	3.67	(1.06-12.74)	0.074	108 (11.6%)	10 (0.9%)	1.69	(0.18-16.11)	< 0.001
Prior abortion	82 (30.1%)	112 (30.5%)	1.06	(0.36-3.14)	0.866	270 (29.03%)	15 (1.4%)	1.22	(0.29-5.17)	< 0.001

Data are expressed as Mean±SD or number (%). OR, odds ratio and CI, confidence interval

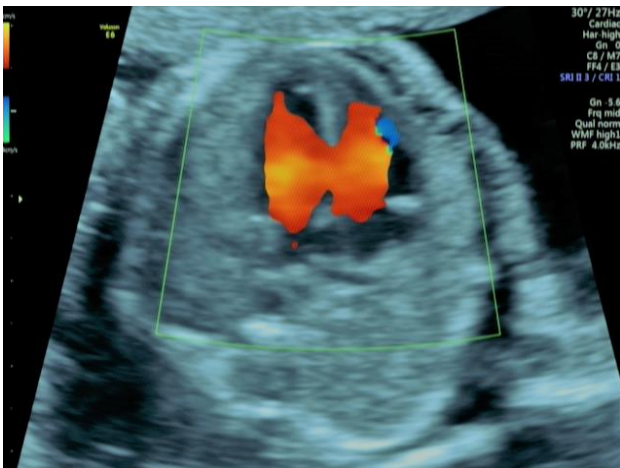


Figure 1: VSD. (4 chamber view with colour doppler image showing +ve flow across the sub valvular interventricular septal defect about 5 mm).

Pregnant women with underlying autoimmune disease without folic acid supplement had a higher peril of total rate of CAs in their progeny (OR: 1.7, 95% CI: 1.1-2.7), excused principally by three isolated CA groups: isolated

renal agenesis or dysgenesis, obstructive CA of the urinary tract (including 2 cases with cystic dysplasia), cardiovascular CA (including 12 cases with ventricular septal defect Figure 1 and 2, but the second most common CA was transposition of great vessels in 1 case) and by the multiple CAs group.



Figure 2: VSD (2D (greyscale) 4 chamber view showing +ve subvalvular VSD).

Table 2: Estimation of different congenital abnormalities (CAs) in the studied groups with or without folic supplementation.

	Folic acid supplementation (N = 648)					No folic acid supplementation				
	Group 1 0.4 mg/day		Group 2 4 mg/day		OR (95% CI)	Group 3 Disease matched- control group No. (630)		Group 4 No past history of methotrexate therapy No (815)		OR (95% CI)
	No. (272)	%	No. (367)	%		No. (930)	%	No. (1015)	%	
Isolated CAs										
Neural tube defects	0	0	0	0.00	- (-)	2	0.21	0	0	2.3 (0.7-7.4)
Cleft lip with or without cleft palate	0	0	0	0.0	0	1	0.10	1	0.09	2.9 (1.0-8.3)
Cleft palate	0	0	0	0.0	0	1	0.10	0	0	3.3 (0.8-13.6)
Renal a/dysgenesis	1	0.36	0	0.00	- (-)	2	0.21	0	0.	24.7 (7.4-82.5)
Obstructive CAs of urinary tract	2	0.72	1	0.27	0.8 (0.2-3.0)	4	0.42	0	0	8.5 (2.6-28.1)
Hypospadias	3	1.1	0	0.0	1.0 (0.4-2.6)	3	0.31	1	0.09	0.7 (0.2-2.7)
Undescended testis	2	0.72	0	0.0	0.6 (0.2-2.7)	9	0.93	1	0.09	0.5 (0.1-3.8)
Ear CAs	0	0	0	0.0	3.9 (0.9-6.2)	8	0.84	0	0.00	- (-)
Cardiovascular CAs	1	0.36	0	0.0	2.1 (1.1-3.5)	11	1.1	1	0.09	2.7 (1.4-5.2)
Clubfoot	0	0	0	0.0	0.8 (0.2-2.5)	13	1.3	1	0.09	0.8 (0.2-3.5)
Poly/syndactyly	1	0.36	0	0.0	0.4 (0.1-2.8)	2	0.21	0	0.0	1.2 (0.3-5.1)
Other isolated CAs	1	0.36	0	0.0	0.5 (0.2-1.7)	7	0.75	1	0.09	0.8 (0.3-2.7)
Multiple CAs	1	0.36	0	0	3.1 (1.4-6.9)	11	1.1	2	0.18	1.4 (0.3-5.90)
Total	12	6.2	1	0.27	1.1 (0.7-1.7)	74	12.3	8	0.7	1.7 (1.1-2.7)

However, the major intent of the study was to inspect whether folic acid supplement (either 0.4 mg or 4 mg/day) 4-5 weeks preconception and for the first 12-13 weeks of pregnancy can reduce the peril of methotrexate-related CA; therefore, pregnant women with underlying autoimmune disease were differentiated into three subgroups: Group 1 who received 0.4 mg/day, Group 2 who received 4 mg/day and Group 3 without folic acid supplement during the study pregnancy. Group 4, pregnant women with no underlying autoimmune diseases or folic acid supplement.

In fetus of pregnant women with folic acid supplementation 4 mg/day pre-conceptional, only one case was affected by obstructive CAs of the urinary tract (Figure 3 and 4). There was no offspring with a neural-tube defect and cleft lip with or without cleft palate, cardiovascular or other anomalies. The total rate of CAs was lower in the fetus of pregnant women in Groups 1

and 2 (OR: 1.1, 95% CI: 0.7-1.7) than in group 3 and 4. There was no offspring with a neural-tube defect or cleft lip±cleft palate in Group 1. One case of renal a/dysgenesis and 2 cases of obstructive CAs of the urinary tract and one case of cardiovascular CAs and multiple CAs in Group 2. The risk of cardiovascular CAs and multiple CAs has remained higher in Group 3 (11 cases for both). However, the total rate of CAs was significantly higher in the fetus of pregnant women without folic acid supplement (OR: 1.7, 95% CI: 1.1-2.7), explained mainly by the higher rate of renal a/dysgenesis, urinary tract obstructive CAs, cleft lip±cleft palate, and cardiovascular CAs. Finally, it would be necessary to appraise the effect of folic acid according to the duration and dose of its supplementation and time of methotrexate therapy stoppage for its cumulative effect, three months before conception may be more effective, therefore, a beneficial preventative effect of folic acid can be more

with a dose of 4 mg/day. Pregnant outcomes were shown in Table 4.

Table 3: Number (%) of cases born to pregnant women with congenital anomalies according to the folic acid supplementation during the study pregnancy.

	Folic acid				No folic acid use				Total
	Group1 0.4 mg/day		Group 2 4 mg/day		Disease- matched group		Cases without underlying autoimmune disease		
	No. (272)	%	No. (367)	%	No. (930)	%	No. (1015)	%	
Isolated CAs									
Neural tube defects	0	0	0	0.00	2	100.	0	0	2
Cleft lip with or without cleft palate	0	0	0	0.0	1	0.50	1	0.50	2
Cleft palate	0	0	0	0.0	1	.100	0	0	1
Renal a/dysgenesis	1	0.33	0	0.00	2	0.66	0	0.	3
Obstructive CAs of urinary tract	3	37.5	1	12.5	4	0.50	0	0	8
Hypospadias	3	42.8	0	0.0	3	42.8	1	14.2	7
Undescended testis	2	16.6	0	0.0	3	25.0	1	8.3	12
Ear CAs	0	0	0	0.0	8	100	0	0.00	8
Cardiovascular CAs	0	0.0	0	0.0	11	91.6	1	8.3	12
Clubfoot	0	0	0	0.0	13	92.8	1	7.2	14
Poly/syndactyly	1	33.3	0	0.0	2	66.7	0	0.0	3
Other isolated CAs	1	9.09	0	0.0	8	72.7	2	18.1	11
Multiple CAs	1	6.25	0	0	14	87.5	2	12.5	16
Total	12	16.1	1	1.01	72	74.7	9	8.08	99

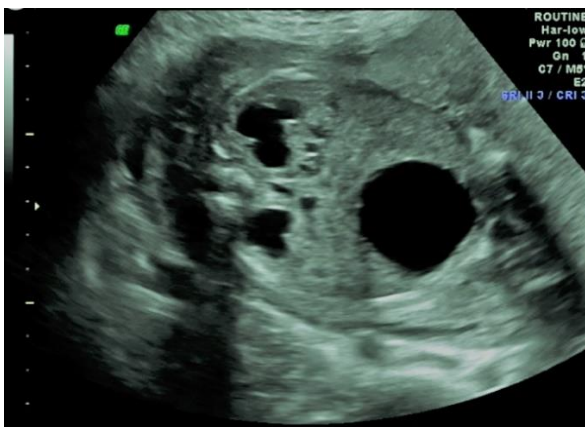


Figure 3: Obstructive urinary tract anomalies. (Grey scale coronal image of the abdomen showing bilateral mild to moderate hydronephrosis and marked distention of U.B.).

The incidences of preterm delivery were higher in the Group 3 and 4 than in the Group 1 and 2 (11.6% versus 1.8%); (OR: 6.8, 95% CI: 1.2-3.2, P < 0.005). Abortion was lower in Group 1 and 2 than others (OR: 2.9, 95% CI: 1.05-8.83) against (OR: 4.39, 95% CI: 2.5-6.5). The incidences of birth weight < 2500 gm was higher in Group 3 and 4 than in Group 1 and 2 (OR: 4.8, 95% CI:

1.8-2.9; p < 0.05). There was no significant difference in umbilical arterial PH. The Apgar scores < 7 at 1, 5 min was higher in Group 1 and 2 than Group 3 and 4 (OR: 4.39; 5.16) versus (2.1; 2.9) respectively. The admission to NICU was higher in Group 3 and 4 than other groups (OR: 9.9, 95% CI: 6.3-5.1) versus (OR: 1.5, 95% CI: 0.8-2.93) also the gestational age at delivery was higher in Group 1 and 2; p < 0.001.

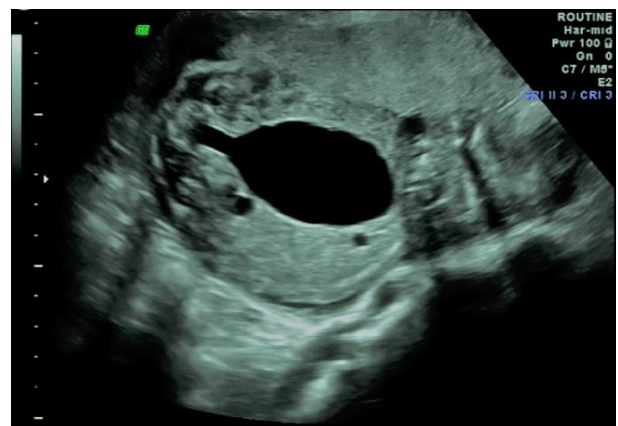


Figure 4: Obstructive urinary tract anomalies. (Grey scale coronal image of the pelvis showing characteristic keyhole deformity, typical for PUV (posterior urethral valve)).

Table 4: Number (%) of cases born to pregnant women with congenital anomalies according to the folic acid supplementation during the study pregnancy.

Study group	Folic acid				No folic acid use				Total No.
	0.4 mg/day		4 mg/day		Disease-matched group		Cases without underlying autoimmune disease		
	N	%	N	%	No.	%	No.		
Isolated CAs									
Neural-tube defects	0	0	0	0	2	100.0	0	0.0	2
Cleft lip with or without cleft palate	0	0	0	0	1	50.0	1	50.0	2
Cleft palate	0	0	0	0	1	100.0	0	0	1
Renal a/dysgenesis	1	25	0	0	2	50.0	1	25.0	4
Obstructive CAs of urinary tract	3	37.5	1	12.5%	4	50.0	1	25.0	8
Hypospadias	3	42.8	0	0	3	42.8	1	19.2	7
Undescended testis	2	16.6	0	0	9	75	1	8.3	12
Ear CAs	0	0	0	0	8	100	0	0	8
Cardiovascular CAs	1	4.5	0	0	21	91	1	4.5	22
Club foot	0	0	0	0	41	97.7	1	2.3	42
Poly/syndactyly	1	28.6	0	0	2	71.4	0	0	7
Other isolated CAs	3	31.25	0	6.2%	7	56.25	1	6.2	16
Multiple CAs	3	30.07	0	0	11	64.28	2	7.6	26
Total	17	14.5	1	1.2	74	78.4	8	5.6	100

Table 5: Comparison between perinatal and maternal outcomes in the studied groups.

	Folic acid					No folic acid				
	0.4 mg/day (N=272)	4 mg/day (N=367)	OR	(95% CI)	p-value	Disease-matched group (N=930)	With no autoimmune disease (N=1015)	OR	(95% CI)	p-value
Admission to NICU	5 (1.8%)	6 (1.6%)	1.5	(0.80-2.93)	0.183	212 (22.7%)	31(3.05%)	9.9	(6.3-5.1)	< 0.05
Gestational age at delivery (weeks)	37.6 (33.9-38.1)	37.6 (34.7-38.4)			0.56	36.7 (25.6-37.7)	37.1 (28.1%)			< 0.001
PTL	5 (1.8%)	0 (0.0%)	2.1	(2.12-21.84)	0.002	108 (11.6%)	60 (5.9%)	6.6	(1.2-3.2)	< 0.005
Birth weight (g)	2786 (1958-3080)	2863 (1290-3740)			0.235	2364 (610-3022)	2778 (1962-3432)			0.002
< 2500 g	5 (1.8%)	1 (0.2%)	3.79	(1.30-11.04)	0.025	112 (12.04%)	70 (6.8%)	4.8	(1.8'-2.9)	< 0.05
Apgar Score 1 min < 7	4 (1.4%)	1 (0.2%)	4.39	(1.05-8.83)	0.08	135 (16.4%)	70 (6.8%)	2.1	(2.5-6.5)	< 0.002
Apgar Score 5 min < 7	2 (0.7%)	0 (0)	5.16	(0.44-60.27)	0.428	95 (10.21%)	13 (1.2%)	2.9	(1.2-2.8)	< 0.002
Umbilical arterial pH	7.330 (7.118-7.493)	7.326 (7.018-7.413)			0.859	7.318 (7.228-7.366)	7.316 (7.057-7.425)			0.815
Preeclampsia	4 (1.4%)	1 (0.2%)	1.07	(0.38-2.96)	0.892	55 (5.9%)	20 (1.9%)	5.1	(2.5-10.5)	< 0.05
Placenta abruption	5 (1.8%)	1 (0.2%)	3.79	(1.30-11.04)	0.025	112 (12.04%)	70 (6.8%)	4.8	(1.8'-2.9)	< 0.05
PROM	4 (1.4%)	1 (0.2%)	2.9	(1.05-8.83)	0.08	135 (16.4%)	70 (6.8%)	4.39	(2.5-6.5)	< 0.002
IUGR	4 (1.4%)	1 (0.2%)	1.07	(0.38-2.96)	0.892	55(5.9%)	20 (1.9%)	5.1	(2.5-10.5)	< 0.05
Abortion	4 (1.4%)	1 (0.2%)	2.9	(1.05-8.83)	0.08	135 (16.4%)	161 (17.3 %)	17.31	(2.5-6.5)	<0.002
IUFD	4 (1.4%)	1 (0.2%)	1.07	(0.38-2.96)	0.892	55 (5.9%)	56 (6.02%)	5.1	(2.5-10.5)	<0.05

Data are expressed as number (%) or median (range), OR (Odds Ratio), CI (Confidence Interval) and NA (Not applicable).

Preeclampsia and placenta abruption were higher in Group 3 and 4 than Group 1 and 2 (OR: 5.1, 95% CI: 2.5-10.5) versus (OR: 1.07, 95% CI: 0.38-2.96) and (OR: 4.8, 95% CI: 1.8-2.9) versus (OR: 3.79, 95% CI: 1.30-11.04). IUGR and IUFD were also higher in Group 3 and 4 (OR: 5.1, 95% CI: 2.5-10.5) versus (OR: 1.07, 95% CI: 0.38 - 2.96). PROM was low in Group 1 and 2 than others (OR: 2.9, 95% CI: 1.05-8.83) against (OR: 4.39, 95% CI: 2.5-6.5).

PTL was lower in Group 2 than Group 1 (1.8% versus 0%), $p = 0.002$. Weight at birth <2500 gm was more in Group 1 than Group 2 (1.8% versus 0.2%), $p = 0.025$. Unfortunately, the numbers were too low for convenient statistical analysis (Table 5).

DISCUSSION

Methotrexate teratogenic effect is known due to its antagonist effect to folic acid. The pre-conceptual low (0.4 mg) doses of folic acid or folic acid (0.8 mg) containing multivitamin, supplementation can reduce significantly the first appearance of neural-tube defects.²²

In addition, as the Hungarian randomized controlled and cohort-controlled trials showed, pre-conceptual folic acid-containing multivitamin supplementation could reduce the occurrence of cardiovascular (especially construal) and urinary tract (especially obstructive) CAs.^{23,24} However, this primary prophylactic method was not able to decrease the multiple CAs occurrence.²⁵ The rating of the population-based case-control dataset in Hungary showed that high doses of folic acid reduced the rate of isolated cardiovascular CAs and oro-facial clefts, but not the occurrence of multiple CAs.^{23,26,27}

Early population studies in the USA suggested that use of multivitamins with folate reduced the risk of non-syndromic NTDs associated with other major birth defects.²⁸⁻³⁰ A major trial of the periconceptual use of folic acid in the prevention of NTDs by a randomized study conducted by the MRC was published in 1991.³¹ From 1981 to 1996 there have been over 10 studies of periconceptual folate use and most of them reported lowered NTDs with folate use.³² Some regions of China have a high incidence of NTDs.³³

The trial in China showed that periconceptual intake of 400µg of folic acid daily significantly decreased the peril of NTDs in areas of both high and low rates of such defects.³⁴ Despite widespread education of the population about folic acid supplementation and food fortification, Kadir and colleagues did not find a decrease in the rate of NTDs in the general population in England and Wales in 1999.³⁵

Shahraki et al, found that, administration of high dose folic acid may reduce the propagation of preeclampsia (PE), there is not sufficient data to favor that higher amount of folic acid administration can reduce the

severity of presentation's signs or ameliorate the laboratory data and the onset of PE.^{36,37} Hernández-Díaz et al, suggested that intake of folic acid during pregnancy may decrease the risk of gestational hypertension.³⁸

However, Li et al, suggested that 400 µg folic acid daily intake only during early pregnancy cannot prevent the occurrence of preeclampsia or gestational hypertension.³⁹

Earlier studies showed that supplement of folic acid was associated with decreased risk of preterm labor; Xiaohui et al, studied the effect of folic acid supplement more than 12 weeks on the peril of preterm birth and found that folic acid usage decreased the peril of preterm birth with a substantial dose-related response.⁴⁰

Bukowski and colleagues reported that pre-conceptual supplement of folate is associated with up to 70% reduction in the incidence of spontaneous early preterm birth which proportionate inversely to the duration of folic acid usage. Pre-conceptual supplement of folate was related to spontaneous early preterm birth and not associated with other pregnancy complications.⁴¹ Wang et al, found that folate supplement decreased the risk of PTL while it was increased in those with abnormal BMI, although cases with higher BMI did not have this high risk when the adjusted analysis was executed. The protective effect of FA supplement for PTL was decreased in cases with BMI ≥ 25 kg/m².⁴²

On the other hand, some studies did not find any benefit for folate supplement in the prevention of PTL; Saccone and Berghella, in their meta-analysis of randomized controlled trials on supplementation of folic acid in pregnancy to prevent PTL, found that folic acid supplementation during pregnancy does not prevent delivery <37 weeks.⁴³ Martinussen et al, found that there was a lack of support for any benefit of folate intake on preterm birth or low birth weight.⁴⁴

Nilsen et al, studied supplementation of folic acid and multivitamin and the risk of placental abruption and suggested that supplementation of folic acid and other vitamins during pregnancy may be associated with decreased risk of placental abruption.^{45,46}

CONCLUSION

In conclusion, methotrexate has a cumulative effect on maternal tissue, so congenital anomalies may appear even after one month of its stoppage before pregnancy. low dose folate is not sufficient to prevent congenital anomalies in those women. High preconception dose (4 mg/day) at least 4-5 weeks and during the first trimester, can prevent anomalies in pregnant women. This can be explicated by the direct prophylactic effect of folic acid against CA, but this possible beneficial effect needs further studies to improve the medical care of those pregnant women.

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