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The Impact of Toxoplasma Gondii Infection on the Vitamin D3 Levels among Women in Childbearing Age, Treatment and Effect on Fetal Brain Development

Sikandar Ali Khan [a] | Erum Rehman [b] | Muhammad Bilal Khattak [c]

Zakia Subhan[d] | Sara Yar Khan [e] | Zahid Irfan Marwat [f]

Abstract: *Pregnant women are at increased risk for contracting toxoplasmosis, a parasitic infection. During pregnancy, the fetal developing brain is especially vulnerable to the stress of an infection, which could result in miscarriage or other complications. The study's goal is to examine how Toxoplasma gondii (T. gondii) infection affects maternal viremia and the mental development of their kids.: In all, about 446 working-age women were asked about their preferences for prenatal care, reproductive care, and urgent care. Beckman is the provider of a 25(OH) VD Access Kit, which may be purchased by customers. To confirm the presence of T. gondii antibodies, blood samples were analysed for levels of 25(OH)D (D3) and ELISA kits were used that were designed specifically for use with humans. They administered a variety of drugs to him and monitored his condition very carefully while doing so. SPSS was used in order to do the analysis of the study's data.: Among the 117 people tested, 31 had IgM antibodies, 49 had IgG antibodies, and 13 had both. Abortion-related proteins were detected in the sera of 65.4% of women who had only one abortion. Most people (98%) were found to have adequate VD (25(OH)D>50 nmol/L) at the time of delivery, with the mean (IQR) 25(OH)D level being 102.1 nmol/L (34-218 nmol/L). It was concluded that Healthy people who are not pregnant generally recover from toxoplasmosis without therapy. Combinations of medications including pyrimethamine, sulfadiazine, and folinic acid may help pregnant women, neonates, and infants feel better, despite the parasite perhaps remaining latent inside tissue cells following therapy. Parasitic illnesses and parasite infections have received little attention in vivo and in vitro studies.*

Key Words: Toxoplasmosis, T. Gondii, Vitamin D, Fetal Development, Brain Development

Introduction

The intracellular apicomplexan parasite T. Gondii can infect many different kinds of animals. Between a third and half of the global population may be infected with the parasite. (Elbahaie *et al.*, 2022). The majority of birds and mammals are categorised as intermediates, making these the much more common hosts for parasites in the world. Due to the egg sacks (oocyst), they leave in

[a] Department of Biochemistry, Khyber Girls Medical College, Peshawar, KP, Pakistan.
[b] Department of Pharmacology, Peshawar Medical College, Warsak Road, Peshawar, KP, Pakistan.
[c] Department of Medicine, Khyber Girls Medical College Peshawar, KP, Pakistan.
Email: Bilalkhattak120gmail.com *(Corresponding Author)*
[d] Department of Pharmacology Institute of Pharmaceutical Sciences Khyber Medical University Peshawar, KP, Pakistan.
[e] Department of Biochemistry, Jinnah Medical College, Peshawar, KP, Pakistan.
[f] Department of Biochemistry, Nowshera Medical College, Nowshera, KP, Pakistan.

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their stool, household and terrestrial cats are endemic species that actively participate in parasite distribution (Al-Qadi *et al.*, 2019). The fact that all these bags are able to withstand extreme environmental factors puts the general public's health in danger. Immediate interaction with programmed cat faeces or consumption of egg bags in food, drink, polluted soils, blood transfusions, organ transplants, or trans-placenta in a container on tissue bags are the two main ways that the disease is spread. In individuals with a healthy immune system, *T. gondii* and its signs are minimal or nonexistent (Kinney *et al.*, 2010; Zhou *et al.*, 2011). Pregnant females who contract a disease pass it on to the fetus, which can result in congenital abnormalities or miscarriage (Al-Qadi *et al.*, 2019).

The VD is mostly produced by the human whereas the skin gets the sunshine, and it occupies a significant place in bones and maintenance by controlling calcium and phosphorus levels of development. VD is also required for the normal functionality of the immune system. VD is a vitamin that would be believed to be one vital biological component to humans, a vitamin soluble in fat, which is present naturally in many foods and is accessible as a nutritional supplement (Deschasaux *et al.*, 2016). The recent decade has seen a significant increase in scientific data supporting VD's significance in central nervous system development and functions (McCann & Ames, 2008). Numerous developmental brain diseases have indeed been indirectly linked to reducing VD levels, either at delivery or in the postnatal period. A pretty strong demographic link suggests that those who go on to develop schizophrenia later in life have somewhat more winter/spring births than usual (Eyles *et al.*, 2011).

The importance of maternal nutrition on fetal development has drawn a lot of attention in recent years. For prenatal cognitive development, which may be the earliest stages in the aetiology of the psychotic spectrum, choline, folic acid, and VD are crucial. Nutritional deficits have been linked to alterations in prenatal brain development. These changes can be seen in initial behavioural and cognitive issues in children, as well as a higher prevalence of psychotic and autistic spectrum disorders in adults (Laird *et al.*, 2017). Pregnant females have a significantly high frequency of VD insufficiency. Additionally, research has shown that reduce maternal VD levels may influence brain development and lead to the emergence of a number of mental diseases, including schizophrenia and autism. Toxoplasmosis has been demonstrated in numerous studies to have an impact on VD. Regardless of the fact that pregnant women have a significant increase in hypovitaminosis-D (Wagner *et al.*, 2013). Epidemiological research is only beginning to show a relationship between low amounts of VD during prenatal and initial postnatal brain development and poor results in brain-related areas. This study was carried out to evaluate the impact of *T. gondii* infection on VD levels among women of childbearing age and to analyze the effect of VD on fetal brain development.

Materials and Methods

Study Design and Area

From January 2018 to August 2022, research lab information was collected after a cross-sectional investigation. The study was carried out at Mardan Medical Complex from Jan 2022 to June 2022. Primary care, obstetrics, contraception, and outpatient care are all available to adult women in the area.

Sample Size and Study Population

The World Health Organization's Practical Guide for the Estimation of Sample Size in Global Health served as the basis for our sample size calculations. Given the paucity of data in this location, we hypothesised that we would need to test at least 245 women with health issues to attain a 95% confidence level, our targeted accuracy of 0.05, and our estimated toxoplasmosis seroprevalence of 50%. Due to the low number of respondents, we increased our original sample size by 10%, for a grand total of 269. Women were selected at random from medical records kept by hospitals linked with universities. The dataset has

information from 446 working women. Women over the age of 18 needing gynaecological, obstetric, or general care from a provider other than a hospital were invited to voluntarily participate.

Data Collection

This information was collected using an online survey that inquired about respondents' ages, marital statuses, income levels, and places of origin. We polled gardeners about their cat ownership, whether or not they ate raw or unwashed meat or vegetables, whether they drank unpasteurized milk or water, and whether or not they washed their hands after handling cat faeces to determine their risk of contracting toxoplasma infection.

Sample Collections and Serum Separation

Each woman in both groups had 5 ml of venous blood drawn after proper antiseptic procedures were taken. The erythro-sedimentation rate can be determined by using the first tube, which contains 0.5 ml of sodium citrate. Blood is drawn and then injected into a second tube containing Ethylene diamine tetra-acetic acid (EDTA) to perform a complete blood count. We transferred the remaining blood sample very carefully into the third tube, which contains a jell-activator that promotes blood clotting and yields clear sera. To prevent blood clots and hemolytic anaemia, tubes 1 and 2 were switched back and forth frequently. Specimens were only kept refrigerated for a maximum of 24 hours in case of delay. After ten minutes, the third tube was centrifuged for five minutes at 3500 RPM. This serum was clear and hemolysis-free, so it was re-contained in a new, sterile plan tube and stored at 30 degrees Celsius.

Serological Detection

Using a commercial human anti-*T. gondii* IgG ELISA kit, respondents' sera were examined for the presence of *T. gondii* antibodies. At 410 nm, optical density (OD) was determined and displayed in IU/L. The proportion of positivity (PP) value was calculated for each sample in relation to the OD of the positive control and used to explain the medical reports. After performing a receiver operating characteristic curve analysis, a PP value of 20 was considered a useful a priori (as recommended by the manufacturer), and a PP value of 20 was considered negative.

Measurement of VD Levels

The Beckman Access 25(OH) VD Kit Assays were used with the Beckman Coulter UniCelDxI 800 Accessibility Immunoassay Technology to quantify the overall serum 25(OH)D (D2 + D3) concentrations in patient serum samples for the present investigation. The overall 25(OH) VD measurement range for Beckman Access is 7–130 ng/mL. 5.7% and 4.5%, respectively, were the intra-assay and inter-assay CVs. A serum 25(OH)D level lower than 30 was considered a deficiency in VD level, between 30 and 49.9 as inadequate, and above 50 as sufficient.

Treatment

A suggested pharmacological treatment combination was provided. It is necessary to schedule frequent follow-up consultations to monitor for adverse effects, visual issues, and general growth.

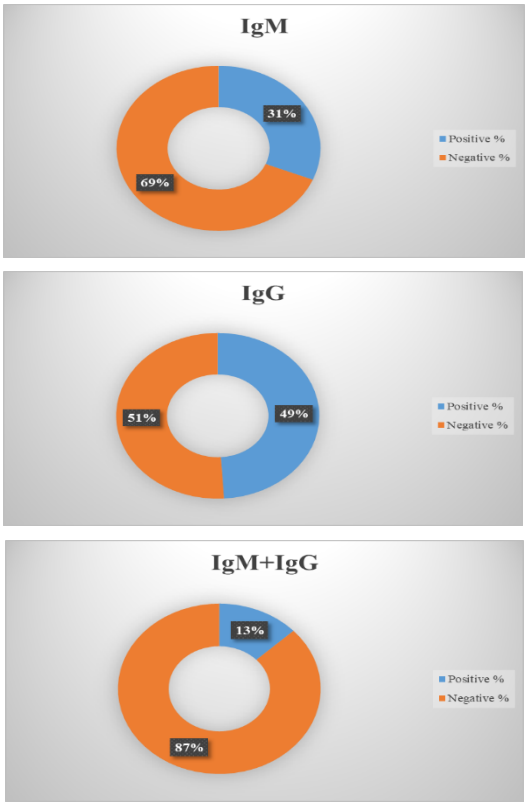
Statistical Analysis

With the aid of a Microsoft Excel file, all gathered information was combined and organised into designated tables. The chi-square and t-student tests for assessing variants and importance at the levels of $P < 0.05$ and $P < 0.01$ were used to examine the difference in the variables between the study and control groups.

Results

The percentage of those who tested positive for toxoplasmosis overall was 72%; the rates for IgM, IgG and IgM + IgG antibodies were 22.87%, 49.01%, and 13.07%, respectively (p 0.05). Out of 446 participants, 117 were found positive for toxoplasmosis and table 1 shows that the incidence of toxoplasmosis was 31% for IgM (figure 1a), IgG

Abs rates varied significantly between the two groups i.e. IgG with 49% (figure 1b) and IgM+IgG having 13% (figure 1c).

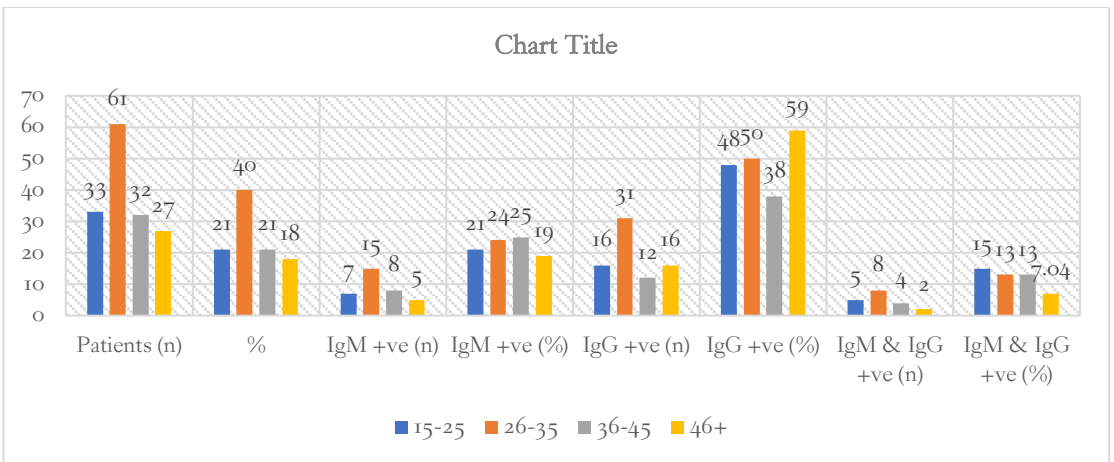


Graph 1: Positive and Negative Percentages of *T. Gondii* Antibody; 1a. IgM, 1b. IgG & 1c. IgM+IgG

Table 1. Summary of the *T. Gondii* Antibody Percentages

Anti Toxoplasma Antibodies	Positive		Negative	
	No.	%	No.	%
IgM	22	35	118	77.13
IgG	75	49.01	78	50.98
IgM+IgG	20	13.07	133	86.93

The frequency of *T. gondii* antibodies is presented for different age groups of females (figure 2). Sera from young mother exhibit higher levels of *T. gondii* IgM than do sera from older mothers (P 0.05). During the first three categories, the IgM levels were 21.21%, 24.59%, and 25%, compared to 18.51% in the sera of females older than 46 years, correspondingly. In contrast to the IgG Abs rate, the sera of women over the age of 46 showed a greater level of 59.25% than did the sera of younger women. Additionally, the proportion of sub-acute cases where both IgM and IgG antibodies were present in the sera was highest among people between the ages of 15 and 25. It was 15.15 per cent in comparison to 7.40 per cent in two samples from women older than 46 years, P 0.05. In the same data, the overall rate of *T. gondii* IgM Abs is 22.87%, compared to 49.01% for IgG Abs.



Graph 2: Frequencies of *T. Gondii* Antibodies in Different Age Groups

Abortion revealed a high positive rate of 64.54% in sera of females who had a single abortion, followed by 41.04% in sera of women who had repeated abortions, and 21.61% in sera of women who already had stillborns, P 0.05, which was used to evaluate the effects of toxoplasmosis on women (table 2). In the present study, 43.13% of abortions were performed on females. IgM Abs from *T. gondii* have a mean of 0.76 IU/ml compared to 1.009 IU/ml for IgG Abs. The analysis also revealed that women with many abortions had a mean IgM Abs concentration that was 0.82 IU/ml greater than those with a single abortion and stillbirth (table 2).

Table 2. In Relation to the Number of Miscarriages and Congenital Defects, Women's Prevalence of *T. Gondii* Antibodies

Outcomes	Positive cases: n (%)	IgM mean SE	IgG mean SE
Single abortion	99 (64.54)	0.76 0.01	1.009 0.13
Multiple abortions	67 (41.04)	0.82 0.02	1.00 0.11
Stillbirth	33 (21.61)	0.61 0.01	1.00 0.15

According to a median (interquartile range) 25(OH)D level of 102.1 nmol/L (34-218 nmol/L), 98.0% of the population had sufficient VD (25(OH)D>50 nmol/L) throughout pregnancy and delivery (Table 3). This percentage falls to 86% if a stricter VD sufficiency threshold of 75 nmol/L is employed. Births occurred at an average of 38.6 weeks gestation and weighed 3,182 grammes. Both women's cognitive test results were within the average range for their ages, and neither woman admitted to using VD pills.

Table 3. Distributions of Maternal Characteristics and 25(OH)D at Different Quantiles

Variables	N	Mean	SD	Min	Max
25(OH)D (nmol/L)	202	102.10	27.30	34.00	218.00
Maternal BMI at enrolment	201	26.38	6.51	16.06	50.03
Delivery weight gain (kg)	201	9.69	4.72	0.10	23.20
Maternal serum DHA (mg/mL)	191	0.03	0.01	0.01	0.05
Maternal serum AA (mg/mL)	191	0.13	0.04	0.03	0.16
Maternal KBIT	191	84.44	14.25	48.00	117.00

Table 4. Fetus Means, SD, and Range for the Demographic, Birth Outcome and Neurocognitive Measures

S. No	Variables	N	Mean	SD	Min	Max
1.	GA (week)	117	38.67	1.35	34	41
2.	BW (gm)	117	3182.70	505	1654	4450
3.	HC (cm)	117	33.56	1.40	30	37
4.	Child age at 5-year test	117	5.54	0.28	5.14	6.32
5.	FT dominant hand	117	23.56	5.41	8.40	37.40
6.	FT non-dominant hand	117	21.40	4.84	9	34.40
7.	PLS total language	117	118.03	5.44	100	128
8.	PLS auditory	117	55.27	2.73	47	60
9.	PLS verbal	117	62.76	3.30	51	68

S. No	Variables	N	Mean	SD	Min	Max
10.	WJ applied problems	117	14.42	3.96	2	23
11.	WJ letter word	117	9.80	5.71	1	24
12.	CBCL total t score	117	59.73	8.67	25	77
13.	KBIT verbal	117	11.44	2.76	6	17
14.	KBIT matrices	117	7.63	1.24	2	9
15.	PROCESS	117	151.59	14.98	116	190

Gestational age = GA, birth weight = BW, finger tapping = FT, preschool language score = PLS, head circumference = HC, birth weight = BW, finger tapping = FT, preschool language score = PLS. We assessed mental acuity with the Woodcock-Johnson (WJ), monitored behavioural changes with the Child Behavior Checklist (CBCL), and determined intelligence with the PROCESS Brief Intelligence Test developed by Kaufman and colleagues in paediatrics. In most cognitive tests, higher scores indicate better performance.

We found no significant association between maternal MeHg exposure or fish diet and VD levels, whether we examined the levels using the untransformed or log-transformed 25(OH)D. Researchers have looked at the effects of 25(OH)D on newborn weight, head size, and neurological sequelae, and their findings are summarised in Table 5. There were no associations between maternal 25(OH)D concentration and birth weight, head size, or cognitive development. There was no correlation between maternal 25(OH)D levels at delivery and any of the predicted key outcomes for cognitive development at age 5 in the unadjusted or adjusted models.

Table 5. Shows the Correlations and Confidence Intervals (95% CI) between 25(OH)D Levels at Birth and Expected Neurocognitive Outcomes at 5 Years of Age

Variables	UA		MA		FA	
	β	95% CI	β	95% CI	β	95% CI
BW (gm)	3.250	(0.745, 5.045)	2.076	(0.452, 4.586)	2.125	(0.472, 4.722)
HC (cm)	0.003	(0.004, 0.012)	0.003	(0.004, 0.012)	0.004	(0.004, 0.012)
DH	0.018	(0.012, 0.054)	0.014	(0.013, 0.043)	0.018	(0.01, 0.046)
FT nondominant hand	0.004	(0.012, 0.013)	0.003	(0.022, 0.03)	0.005	(0.023, 0.033)
TL	0.02	(0.03, 0.02)	0.013	(0.014, 0.042)	0.018	(0.01, 0.046)
A	0.006	(0.02, 0.021)	0.006	(0.007, 0.021)	0.008	(0.006, 0.022)
V	0.004	(0.012, 0.024)	0.005	(0.012, 0.024)	0.009	(0.009, 0.027)
WJAP	0.002	(0.023, 0.021)	0.004	(0.017, 0.023)	0.005	(0.015, 0.025)
WJLW	0.007	(0.03, 0.021)	0.002	(0.029, 0.019)	0.007	(0.031, 0.017)
CBCL	0.003	(0.048, 0.045)	0.006	(0.052, 0.038)	0.011	(0.058, 0.036)
KBIT verbal	0.011	(0.027, 0.005)	0.01	(0.024, 0.004)	0.009	(0.023, 0.005)
KBIT matrices	0.002	(0.008, 0.004)	0.002	(0.008, 0.004)	-0.003	(0.009, 0.003)

BW: Birthweight, HC: Head circumference, DH: dominant hand, TL: total language, A: auditory, V: verbal, WJAP: applied problems, WJLW: letter word, CBCL: total t score, UA: Unadjusted, MA: Minimally adjusted and FA: Fully adjusted.

If foetal infection is proven (i.e., by a positive result of amniotic fluid PCR) or highly suspected, we advise treating pregnant women with pyrimethamine, sulfadiazine, and folinic acid (e.g., due to foetal abnormalities consistent with congenital toxoplasmosis detected during ultrasound examination). Initially, 60 participants were divided into two groups of 30. (table 6). In contrast to VD-sufficient people, those who lacked

the gene were more likely to get Toxoplasma gondii (table 7). Toxoplasmosis may resolve itself in some otherwise healthy people who aren't attempting to conceive. It is possible to treat individuals with a combination of medications, including folic acid, pyrimethamine, and sulfadiazine. The parasite can't be cured, although treatment is available for pregnant women, neonates, and infants. Since the parasites may become dormant inside the tissue cells, it may be difficult to entirely destroy them with the treatment. There is a need for a greater study to better understand the in vivo and in vitro interpretations of VD and parasite infections.

Table 6. We See that Groups A and B had very Similar Median Values for VD Concentration (ng/ml) and Toxoplasma Seropositivity (%).

Group	No. of Participants	Type	VD Average Concentration(ng/ml)	Toxoplasma Seropositivity (%)
A	30	VD deficient	9	30
B	30	Normal vitamin D	65+	15

Table 7. The Average Concentration of VD (ng/ml) and Percent of Toxoplasma Seropositivity in Age Groups

	Group A			Group B		
Age	No.	VD average concentration (ng/ml)	Toxoplasma seropositivity No. (%)	No.	VD average concentration (ng/ml)	Toxoplasma seropositivity No. (%)
18-30	10	6.36	1(3.2)	15	49.32	1(3.2)
31-40	13	7.81	6(16.82)	11	60.87	0
41-50 or above	7	10.53	3(8.85)	4	48.11	1(3.2)

Discussion

T. gondii seroprevalence screening has historically enhanced maternal and infant health when performed on all women (Robert-Gangneux et al., 2012; Rasheed et al., 2021). Even though it's common knowledge that pregnant women are more vulnerable to infection and have a higher immune response to certain viruses, the processes behind these changes are still poorly understood (Sappenfield et al., 2013; Kourtis et al., 2014; Fitzgerald et al., 2020). Toxoplasmosis was discovered in 19.4% of women in different research conducted in Pakistan, whereas 72.0% of participants in the present study tested positive. It became out that this was the case (Nazir et al., 2017). Cenci-Goga et al. (2011), Mirza Alizadeh et al. (2018), and Kim et al. (2022) all found that as many as one-third of the global population may be infected with T. The incidence of toxoplasmosis varies substantially from one area to another. Toxoplasma gondii positivity was discovered in just 18.8% of Saudi females in a recent investigation (Rasheed et al., 2021). The prevalence of toxoplasmosis among Moroccan women was 43%, according to a separate study. To wit: (Laboudi et al., 2021). Another study conducted in Annaba, Algeria, found that 47.8% of pregnant women had toxoplasmosis (Messerer et al., 2014). According to recent studies, the seroprevalence of toxoplasmosis among women in Dhamar (Yemen) is 21.2%. The findings were published in 2019 (Al-Adhroey et al. 2019).

Toxoplasmosis is an anthropozoonic disease brought on by the protozoan parasite Toxoplasma gondii (Al-Qadi et al., 2019). Taken combination with the women's year-round exposure to UVB and their very high fish diet, the fact that more than 98% of pregnant women in an equatorial area had acceptable VD status (>50 nmol/L) at birth is important to keep in mind. However, we found no associations between maternal 25(OH)D levels as high as 218 nmol/L and their children's birth

weight, head size, or cognitive outcomes at age 5. feedback (constructive or critical) Multiple additional research has come to the same conclusion as ours: VD does not affect the developing brain. Paternal 25(OH)D levels (interquartile range: 45.2-90.4 nmol/L) were not associated with neurocognitive outcomes (such as IQ or reading ability) in a study of 7065 mother-child pairings in southwest England (>4 to 9 years old) (Darling et al., 2017). In addition, neither Strm et al. (2014) nor Gale et al. (2015) found any connections between parental 25(OH)D status >75 nmol/L with indicators of cognitive development in 9-year-old British children. Several researchers (Gale et al., 2008) In contrast to the findings of Morales et al. (n = 1820; maternal 25(OH)D interquartile range: 54.4-93.1 nmol/L), found a positive linear link between maternal VD level and their children's cognitive and motor development, the current investigation found no such association. Infants born to pregnant Australian women with VD levels of 46 nmol/L or below had double the risk of having delayed language development compared to infants born to mothers whose 25(OH)D levels were over 70 nmol/L. The statements of Whitehouse and company (2012).

If PCR testing of amniotic fluid at 18 weeks of gestation or later reveals foetal infection, treatment with pyrimethamine, sulfadiazine, and folinic acid has shown favourable benefits in the current study and is indicated. As the mother's focus switches from her own physiological requirements to those of her growing baby, her blood 25-hydroxyvitamin [25(OH)D] levels decline. Vitamin D supplementation during pregnancy has been associated with improved maternal and infant health (Pérez-López et al., 2020). This is why it is recommended that all mothers-to-be take 600 IU of vitamin D3 each day. Increased VD dosages (1000-4000 IU/day; Pérez-López et al., 2020) may enhance maternal and newborn outcomes, according to certain research. VD supplementation during pregnancy is favourable for maternal, foetal, and, immediate and later child health, according to recent data from VD intervention trials and meta-analyses of a large amount of research. Some of the prenatal risk factors for aberrant cognitive development may be reduced if more help is given to families in lower socioeconomic groups. In the long run, these efforts may help future generations by making assistance more efficient generally.

Conclusion

Of 117 women who had just one abortion, 64% had positive abortion tests in their sera. The median (interquartile range) 25(OH)D level was 102.1 nmol/L (34-218 nmol/L) at birth, indicating that virtually all participants (98%) had adequate vitamin D levels (25(OH)D>50 nmol/L) at birth. Neither the unmodified nor the covariate-adjusted models identified a correlation between the birth mother's 25(OH)D level and her child's birth weight, head size, or cognitive development. All of the women scored at or above the median on the neuropsychological exams, and none of them reported ever having taken VD tablets. Toxoplasma gondii infection was less common in individuals with sufficient VD levels compared to those who lacked it. Healthy people who are not pregnant generally recover from toxoplasmosis without therapy. Folic acid, pyrimethamine, and sulfadiazine were frequently prescribed together by doctors. Although the parasite's medical condition may be addressed, it will not go gone entirely. As the parasites might possibly hibernate in the cells of the damaged tissues, they may be difficult to eliminate. Parasitic illnesses and parasite infections have received little attention in in vivo and in vitro studies.

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