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Peri-conception Nematode Parasitosis Is an Ecological Problem but Solely Is Not a Risk for Complicated Pregnancy

Youssef Abdel Zaher[1], Marwa Mohamed Naguib[2], Emad M Abdelrahman[3], Dina AbdelhadyMohammed[2], Hamasat Abdelhafiz Elnoury[4] Ali A. Morsy[1]

[1]Department of Obstetrics and Gynecology, [2]Department of Medical Parasitology, [3] General Surgery Department, [4]Department of Clinical and Chemical Pathology, Faculty of Medicine, Benha University

Corresponding Author: Emad M. Abdelrahman, Email: emadshan301@gmail.com.

Tel: O1226763986, ORCID https://www.orcid.org/0000-0001-6066-2215

ABSTRACT

Background: Nematodes represent a continuous worldwide challenge with formidable burden on health and economics.

Objective: The study aimed to assess the prevalence of nematodes parasitosis (NP) among pregnant women and its relation to the pregnancy-associated complications (PACs).

Patients and Methods: In the current study 792 pregnant women were clinically evaluated and gave blood samples and three stool specimens per woman for parasitological evaluation. All women attended the follow-up visits and gave labor at the hospital. The frequency of PACs was determined and its relation to NP was examined statistically.

Results: Multiple nematode species were detected in 185 specimens with A. lumbricoides, E. vermicularis, and T. trichiura represented 76.2% of the detected parasites in varied combinations, while T. spiralis was the least and was detected using human anti-trichinella spiralis IgG ELISA kit. The incidence of PACs was significantly higher among infected women with anemia was the commonest. The frequency of low hemoglobin concentration (HBC) was significantly higher, the average HBC was significantly lower, and the frequency of moderate and severe anemia was significantly higher among the infected women. The number of women who had instrumentally aided vaginal delivery or operative delivery was significantly higher among infected women.

Conclusion: Nematode parasitosis is an ecological problem and is frequent among pregnant women especially those dependent on outdoor food staffs. Multiple-families NP is significant predictor for upcoming PACs. NP in obese pregnant women especially older ones has more deleterious impact on pregnancy.

Keywords: Pregnancy-associated complication, Nematode parasitosis, Women's constitutional characters, Type of work, Residence

INTRODUCTION

Nematodes represent a continuous worldwide challenge with formidable burden on health and economics, where about 24% of the world population, being infected with at least one nematode [(1)]. The persistence and worldwide high infection rate by nematodes were recently attributed to epigenetic changes in female oocytes in the form of altered gene and microRNA expressions thus affecting the phenotype through intergenerational or trans-generational mechanisms [(2)].

Unfortunately, children and pregnant women carry the heavier pathological burden, and disease caused by nematodes especially the blood-feeding worms resulting in anemia, malnutrition and growth stunting with delays in physical and intellectual development and adverse pregnancy outcomes [(3)].

The deleterious impacts of the incident nematode infection during the peri-pregnancy period were attributed, using animals' models, to maternal immunity relaxation with reduction of acquired immunity to gastrointestinal nematodes especially during late pregnancy and lactation [(4)], through reduction of the intestinal epithelial γδ T cells, which play an important role in controlling infection with intestinal nematodes and limiting infection-induced pathology [(5)]. Another hypothesis was the alteration of maternal microbiome secondary to maternal infection by gastrointestinal nematodes [(6)]. A recent animal study assured the hypothesis of maternal peri-parturient relaxation of immunity and attributed it to protein malnutrition and assumed correction of maternal local intestinal immunity by the provision of high protein diet [(7)]. This clinical observational study tried to evaluate the prevalence of nematodes infections among pregnant women and its relation to the pregnancy's outcomes.

PATIENTS AND METHODS

Study design

The current study was conducted at the Departments of Clinical Parasitology, Obstetrics and Gynecology, and Clinical Pathology, Faculty of Medicine, Benha University. Throughout the period from October 2022 till October 2023

Inclusion criteria: Pregnant women, who attended the antenatal care (ANC) unit early in pregnancy, gave blood samples and stool specimens, gave labor at BUH, and were free of exclusion criteria were enrolled in the study.

Exclusion criteria: Maintenance on immunosuppressive therapy, autoimmune diseases, essential hypertension, diabetes mellitus, chronic renal failure, and evident liver dysfunction were the exclusion criteria. Women who refused to participate in the study, lost during follow-up, and had delivery outside BUH were also excluded.

Study Protocol

A) Preliminary evaluation:

The study entails evaluation of the pregnant women at their 1st attendance to the antenatal care unit (ACU) at

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Benha University Hospital (BUH) for data collection concerning age, residence, educational level, work, economic status, special habits, dietary pattern, type of water supply in their residence region, and history of previous or current parasitic infestation, treatment for any parasitic infestations, visiting any of areas known to have endemic parasitosis. Thereafter, patients were asked to attend Clinical Pathology outpatients' lab fasting at least for 6-h to give urine and fresh stool samples and blood samples for conveying the required investigations.

B) Obstetric evaluation

Obstetric history including history of pregnancy-associated complications (PAC), modes of delivery and outcomes of previous pregnancies, and number of living offspring was obtained. Weight and height were determined for the calculation of body mass index (BMI) as weight divided by squared height in meter (kg/m^2) **[8].** and BMI was graded according to **WHO [9] .** recommendations as underweight (BMI<19.9), average (BMI=20-24.99), overweight (BMI=25-29.99) and obese if BMI was ≥30 kg/m^2. Then, all women were examined using abdominal US (AUS) and/or transvaginal US (TVU) for uterine abnormalities and number of gestational sacs.

C) Parasitological evaluation

All women were asked to present three stool specimens collected at intervals of 2-3 days. Stool specimen collection and processing was performed according to the instructions of the Centers for Disease Control and Prevention (CDC); Division of Parasitic Diseases and Malaria**.** Stool specimens were collected in a dry, clean, and leakproof container and must be free of water, urine or contaminants. Unpreserved specimens were refrigerated at 3-5°C in an air-tight container as soon as possible until being processed **[10].** Preferably, specimens were divided into two parts and stored in 10% formalin and polyvinyl-alcohol (PVA) to take advantage of both preservatives. Preservation was performed in a volume ratio of 1:3; fecal material to preservative to prevent sample deterioration**.**

Specimens processing and examination were performed using the Mini-FLOTAC technique with the Fill-FLOTAC for diagnosis of helminths infection as previously described by **Cringoli *et al.* [11]** and modified by **Maurelli *et al.* [12].** Briefly, 38 ml of floatation solution were put in the Fill-FLOTAC stool container, the conical collector was filled with about 2 g of feces using the supplied spatula and the surface was leveled and the Fill-FLOTAC was tightly closed and sample was homogenized by the homogenizer in the cap of the Fill-FLOTAC. Then, a tip was put onto the Fill-FLOTAC, the suspension was turned up-down and the tap was used to fill both chambers of the Mini-FLOTAC through the filling holes until a small meniscus was formed and to avoid the formation of air bubbles, the Mini-FLOTAC apparatus was held at a slope of about 30°. After 10 minutes, the reading disc was applied and rotated clockwise by the key to provide 4 films. Lastly, the Fill-FLOTAC was put under the microscope using the microscope adaptor and was read using the maximal permissible magnification (x400). The multiplication factor to get the number of eggs/larvae/oocysts/cysts per gram of feces was 10.

D) Hematological evaluation

Blood samples were obtained under complete aseptic conditions and were divided into multiple tubes according to type of lab analysis:

1. Sodium fluoride tubes for estimation of baseline blood glucose level by glucose oxidase method **[13].**
2. Ethylenediaminetetraacetic acid (EDTA) containing tube (about 1.8 mg tris EDTA/ 1 ml blood) for at once hemoglobin concentration (HBC) estimation by cyanomethemoglobin method. Gestational IDA was defined as previously documented by **Api *et al.* [14]** according to the trimester, as HBC <11 g/dl in 1st or 3rd trimester and as HBC<10.5 g/dl in the 2nd trimester.
3. Plain tube to allow blood to clot and then centrifuged at 3000 rpm for 10 minutes to separate serum that was collected in a sterile Eppendorf tube and stored at -80°C till be assayed for human anti-trichinella spiralis IgG using ELISA kit (Cat. No. ab108780, abcam Inc., San Francisco, USA) according to the manufacturer instructions **[15].**

Another blood sample was obtained to prepare peripheral blood smear (PBS) by spreading a drop of blood thinly onto a glass slide and stained with coloring agents such as Giemsa stain **[16]** to assess the size, shape and color of RBCs. Accordingly, anemia was classified into hypochromic microcytic, normochromic normocytic, and macrocytic hyperchromic anemia with iron deficiency anemia (IDA) was the main constituent of the hypochromic microcytic anemia; provided absence of morphological changes **[17].**

E) Follow-up

All women were asked to attend the ACU regularly at the start of each trimester for clinical re-evaluation, on developing any clinical manifestations of PAC especially bleeding, and for delivery.

Ethical consideration:

The current study was conducted after approval of the Ethical and Research Committee, Benha University (RC:4-8-2023). Informed consent was obtained from the included patients. This study was executed according to the code of ethics of the World Medical Association (Declaration of Helsinki).

Statistical analysis: SPSS, version 25 (IBM Corp., Armonk, New York, USA) was used for statistical analysis. The χ^2 test was used for qualitative parameters, which were presented as frequency and percentage. Student t-test was used for quantitative parameters, which were presented as mean + standard deviation (SD). The confidence interval was set to 95% and the margin of error accepted was set to 5 %. P values of less than 0.05 were considered significant.

RESULTS

Only 63 pregnant women were out of inclusion requirements, 32 women were missed during preliminary evaluation, 65 were lost during follow-up and 52 women gave labor outside BUH. Parasitic infestation; current or ex-infestation, was detected in 587 women (74.1%) and were collected as Infected group, while parasitic infestation was excluded in 205 women (25.9%) and were grouped as the Uninfected group (Fig. 1).

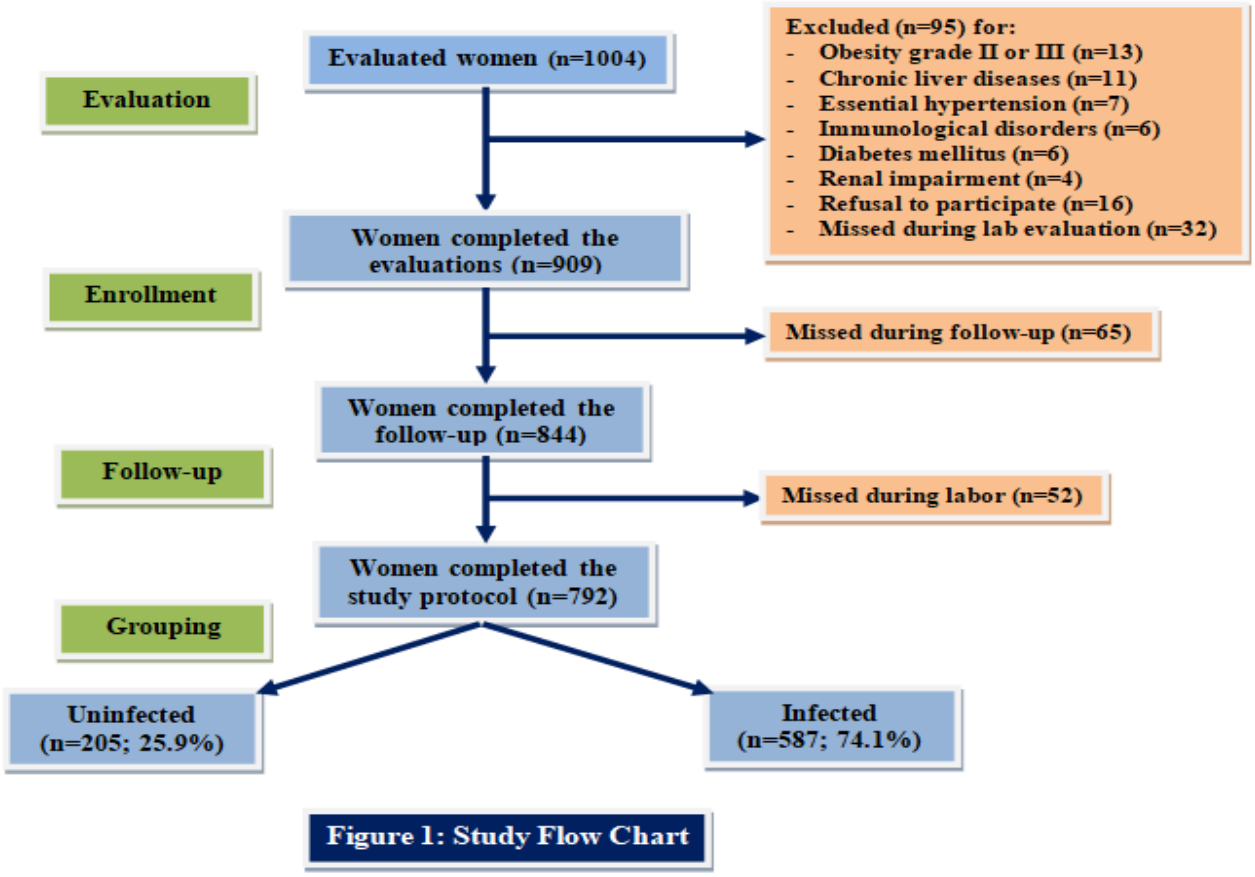

Figure 1: Study Flow Chart

Mean age of the enrolled women was 27.9 (±3.2) with non-significant difference between women of both groups. However, the frequency of women older than 30 years was significantly lower among the uninfected women. The mean BMI was significantly lower among infected women. Despite of the non-significant difference between women according to their residence, the frequency of civilians was significantly higher, while the frequency of rustics was significantly lower among infected women (Table 1).

Table 1: Patients' demographic and residence data

Data	Group	Uninfected (n=205)	Infected (n=587)	P
Age	20-25	31 (15.1%)	103 (17.6%)	0.025
	26-30	131 (63.9%)	407 (69.3%)	
	>30	43 (21%)	77 (13.1%)	
	Average (±SD)	28.2 (2.8)	27.8 (2.9)	0.087
BMI	Average weight (<24.9)	3 (1.5%)	41 (7%)	0.002
	Overweight (25-29.9)	157 (76.5%)	456 (77.7%)	
	Obese-I (30-34.9)	45(22%)	90 (15.3%)	
	Average (±SD)	29.1 (1.9)	28.64 (2.15)	0.007
Residence	Rustic	70 (34.1%)	156 (26.6%)	0.039
	Semi-urban	51 (24.9%)	137 (23.3%)	0.084
	Ex-urban	28 (13.7%)	79 (13.5%)	
	Civilian	56 (27.3%)	215(36.6%)	0.016

BMI: Body mass Index. SD: Standard deviation

Distribution of the enrolled women according to their educational level showed non-significant difference between both groups. There was significant difference between infected and uninfected women as regards type of work; about 25% of the studied women were housewives with significantly (P=0.009) higher frequency among uninfected women, interestingly, the frequency of officers and university staff was significantly (P=0.031) higher among infected (36.1%) than uninfected women (27.8%) (Table 2).

Table 2: Patients' social data

Data	Group	Uninfected (n=205)	Infected (n=587)	P
Education	Illiterate	29 (14.1%)	114 (19.4%)	**0.342**
	Primary school	37 (18%)	93 (15.8%)	
	Secondary school	42 (20.5%)	137 (23.3%)	
	High school	51 (25%)	117 (20%)	
	University graduate	37 (18%)	95 (16.2%)	
	Postgraduate	9 (4.4%)	31 (5.3%)	
Work	Housewives	66 (32.2%)	137 (23.3%)	**0.015**
	Farmers	42 (20.5%)	91 (15.5%)	
	Workers	40 (19.5%)	147 (25%)	
	Officers	45 (22%)	158 (26.9%)	
	University staff	12 (5.9%)	54 (9.2%)	
Annual income	Low	42 (20.5%)	183 (31.2%)	**0.0003**
	Moderate	109 (53.2%)	214 (36.5%)	
	High	47 (22.9%)	159 (27.1%)	
	Very high	7 (3.4%)	31 (5.3%)	

There were non-significant differences between women of both groups as regards number of gravidity and parity. According to the number of living offspring of multigravidas, there was non-significant variance between both groups. The frequency of women who had unaided vaginal delivery was significantly higher, while the frequency of women who had operative delivery was significantly lower among infected women (Table 3).

Table 3: Patients' obstetric data

Data Group			Uninfected (n=205)	Infected (n=587)	P
Gravidity	Primigravida		63 (30.7%)	221 (37.6%)	**0.075**
	Multigravida		142 (69.3%)	366 (62.4%)	
Parity	Primipara		26 (18.3%)	59 (16.1%)	**0.553**
	Multipara		116 (81.7%)	307 (83.9%)	
	Total		142 (69.3%)	366 (62.4%)	
Previous PAC	Frequency	No	75 (52.8%)	154 (40.1%)	**0.029**
		Yes	67 (47.2%)	212 (59.9%)	
		Total	142 (100%)	366 (100%)	
	PACs	Miscarriage	23 (16.2%)	84 (23%)	**0.725**
		Gestational hypertension	20 (14.1%)	65 (17.8%)	
		Gestational diabetes mellitus	16 (11.3%)	38 (10.4%)	
		Antepartum hemorrhage	8 (5.6%)	25 (6.8%)	
		Total	142 (100%)	366 (100%)	
Number of living offspring	No		39 (27.5%)	78 (21.3%)	**0.482**
	One		53 (37.3%)	131 (35.8%)	
	Two		34 (23.9%)	107 (29.2%)	
	Three		14 (9.9%)	41 (11.2%)	
	More than three		2 (1.4%)	9 (2.5%)	
	Total		142 (100%)	366 (100%)	
Mode of the last delivery	Unaided vaginal delivery		32 (24.8%)	131 (38.8%)	**0.005**
	Instrumental vaginal delivery		41 (31.8%)	95 (27.9%)	**0.434**
	Operative delivery		56 (43.4%)	112 (32.8%)	**0.039**
	Total		129 (100%)	338 (100%)	

Collectively, the total number of parasites detected on specimens' examinations was 786 parasites for a rate of 1.34 parasite/specimen. Specimens' examinations detected >2 parasites/specimen in 14 specimens (2.39%), 2 parasites/specimen in 171 specimens (29.13%) and 402 specimens (68.48%) showed one parasite (Fig. 2).

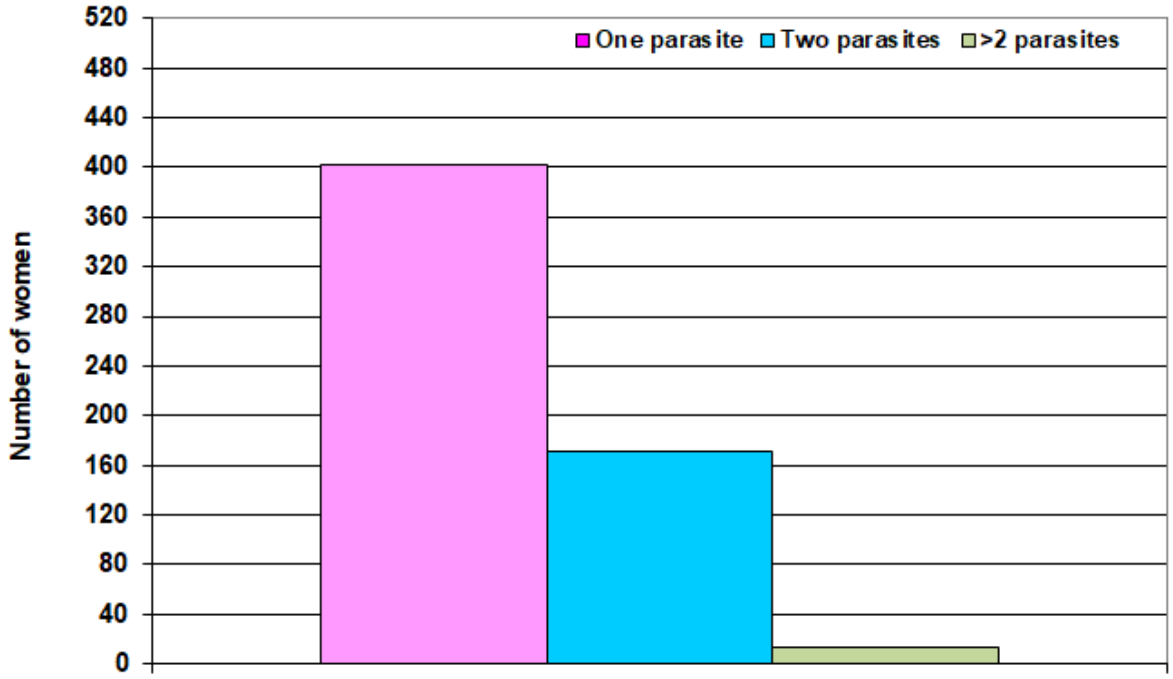

Fig. (2): Infected women's distribution according to number of parasites detected in each specimen

A. lumbricoides, E. vermicularis and T. trichiura were the commonest and represented 76.2% of the detected parasites in varied combinations. A. duodenale and S. stercoralis were the next, while T. spiralis was the least to be found (Fig. 3).

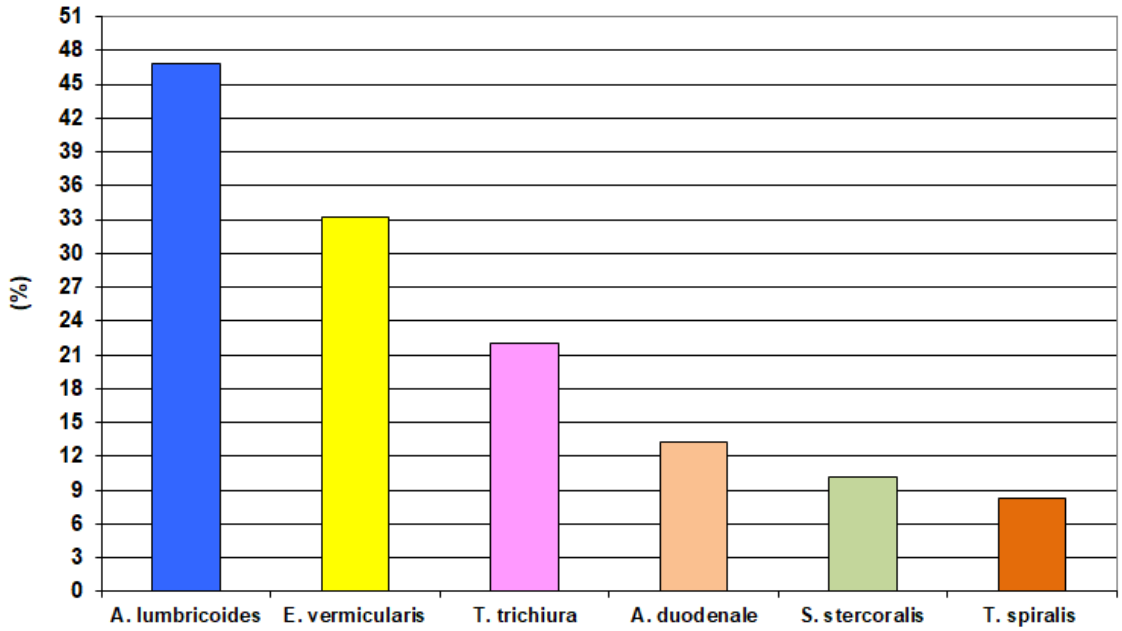

Fig. (3): Distribution of infected women according to type of the detected parasite

During the follow-up period of the included women, 552 PACs were encountered; the incidence of PACs among women of the infected group was 422 PAC (71.9%) and was significantly higher than that reported for women of the uninfected group (n=130; 63.4%).

Anemia was the commonest PAC encountered in both groups for an incidence of 44.4% and was significantly higher among the infected women; 46.5 vs. 38.5. Emesis gravidarum was the commonest medical problem and showed insignificantly higher incidence with parasitic infestation. Pregnancy-related bleeding was reported in 52 women; 14 and 38 in uninfected and infected women, respectively with non-significant difference between both groups. Fifty-nine fetal problems (7.4%) were recorded with insignificant difference between infected and uninfected women as shown in table 4.

Table 4: The pregnancy-associated complications rates encountered in both groups

Complication	Group	Uninfected (n=205)	Infected (n=587)	P
Anemia		79 (38.5%)	273 (46.5%)	**0.048**
Medical problems	Emesis gravidarum	17 (8.29%)	49 (8.35%)	**0.909**
	Gestational hypertension	3 (1.46%)	7 (1.19%)	
	Gestational diabetes mellitus	4 (1.95%)	9 (1.53%)	
	Total	24 (11.7%)	65 (11.1%)	**0.805**
Bleeding problems	Miscarriage	3 (1.46%)	10 (1.7%)	**0.948**
	Spotting	3 (1.46%)	9 (1.53%)	
	Missed abortion	2 (0.98%)	7 (1.19%)	
	Placental abruption	2 (0.98%)	5 (0.85%)	
	Postpartum bleeding	4 (2%)	7 (1.19%)	
	Total	14 (6.83%)	38 (6.47%)	**0.860**
Fetal problems	Intrauterine growth retardation	3 (1.46%)	27 (4.6%)	**0.256**
	Polyhydramnios	1 (0.49%)	31 (5.3%)	
	Premature Preterm Rupture of Membrane	2 (0.98%)	6 (1%)	
	Preterm labor	3 (1.46%)	11 (1.9%)	
	Small-for-gestational age	4 (1.95%)	33 (5.6%)	
	Total	13 (6.34%)	46 (7.84%)	**0.483**
Total		130 (63.4%)	422 (71.9%)	**0.023**

There was a significantly higher number of women had low HBC among the infected women. Further, the average hemoglobin concentration was lower in samples of infected women than uninfected women. Among women who had low HBC, there was significant higher variance between women of both groups as regards the severity of anemia and there was significantly (P=0.004) higher incidence of moderate and severe anemia among the infected women. There was significant variance between infected and uninfected women according to type of anemia and significantly (P=0.0134) higher incidence of IDA among uninfected than infected women (Table 5).

Table 5: Patients' distribution according to severity and type of anemia

	Group		Uninfected (n=205)	Infected (n=587)	P
Hemoglobin concentration (g/dl)	**Frequency**	Normal	126 (61.5%)	314 (53.5%)	0.048
		Low	79 (38.5%)	273 (46.5%)	
	Average (±SD)		11.1 (1.1)	11 (1.15)	0.279
Severity	**Mild**		61 (77.2%)	163 (50.2%)	0.017
	Moderate		13 (16.5%)	81 (24.9%)	
	Severe		5 (6.3%)	29 (8.9%)	
Type	**Microcytic hypochromic**		60 (75.9%)	166 (60.8%)	0.019
	Macrocytic hyperchromic		9 (11.4%)	71 (26%)	
	Normocytic normochromic		10 (12.7%)	36 (13.2%)	

As regards mode of delivery, 443 women (55.9%) had unaided vaginal delivery with significantly lower frequency among the infected women (52.1%) than among women of the free group (66.8%). The frequency of women had instrumentally-aided vaginal delivery or operative delivery was significantly higher among infected than uninfected women (Fig. 4).

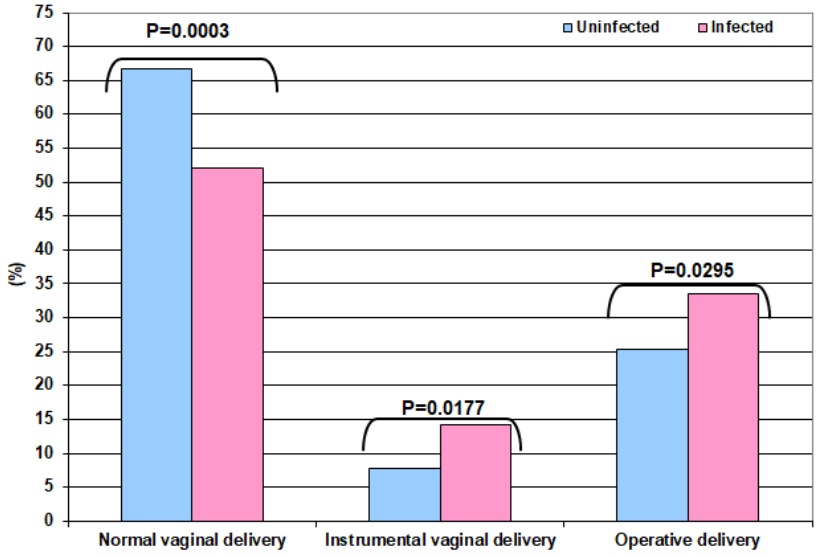

Fig. (4): Distribution of women of both groups according to mode of delivery

Analysis of patients' constitutional data, presence of parasitosis, and multiple parasitosis as predictors for the possibility of PAC development using the ROC curve analysis as judged by the significance of area under the curve (AUC) in relation to area under the reference line arranged these variates as multiple parasitosis, obesity, older age as the significant predictors, while excluded just parasitosis as a predictor (Fig. 5). Using multivariate regression analysis of variates with significant areas excluded age as predictor and assured the utilization of multiple parasitosis and obesity as predictors for upcoming PAC (Table 6).

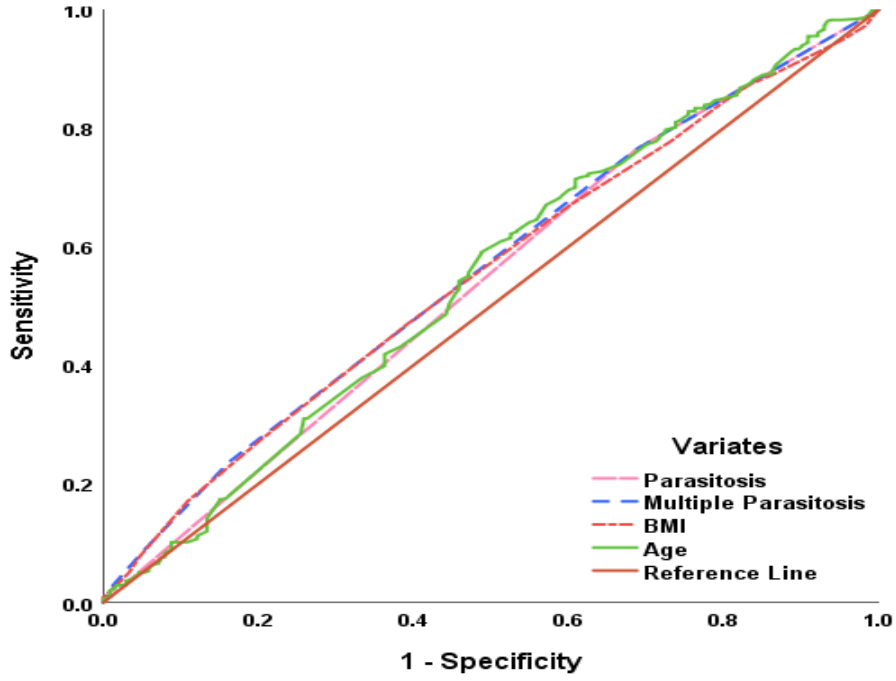

Figure 5: ROC curve analysis for early prediction of upcoming PAC

Table 6: Statistical analyses for early predictors for upcoming PAC

Variates	Receiver Operating Characteristic Curve				Regression analysis	
	AUC	SE	P	95% CI	B	P
Parasitosis	0.538	0.023	0.085	0.494-0.583	Excluded	
Multiple parasitosis	0.559	0.022	0.008	0.516-0.602	0.108	0.002
Age	0.547	0.023	0.035	0.503-0.592	Excluded	
BMI	0.553	0.022	0.018	0.510-0.596	0.081	0.022

PAC: Pregnancy-associated complications; BMI: Body mass index; AUC: Area under curve; CI: Confidence interval; β: Standardized coefficient, SE: Standard error.

DISCUSSION

The detected prevalence of intestinal parasites in specimens of the studied pregnant women was 74.1% with an infection rate of 1.34 parasites per specimen. This infection rate indicated the multiplicity of infecting parasites per patient and spotlights an important ecological problem that parasitic infestation is still endangering humans despite the progress in civilization and governmental provision of means of improved quality of living conditions especially for civilians and ex-rural individuals. Unexpectedly, the frequency of civilians was found to be significantly higher, while that of rustics was significantly lower among the infected women. Moreover, the frequency of housewives and farmers was higher, while the frequency of workers, officers, and university staff was lower among uninfected women.

This discrepancy might be attributed to the dependence of high percentages of women who follow scheduled working times on outdoor prepared foods that were supplied by delivery channels, where perfect cleansing of food staffs especially vegetables is questionable with special regard to sticky ova as in case of ascariasis and because of the dependence on grilled and semi-prepared foods, which is incapable of full eradication of infecting meat-bone parasites. In support of this assumption, A. lumbricoides was detected in about 50% of studied specimens and T. spiralis was detected in about 8.3% of specimens, despite of the low frequencies of pork-transmitted parasites among Muslim persons. This finding indicated the illegal use of pork meat through the semi-prepared meat in the form of packaged minced meat, hamburgers, sausages even if used for homemade food preparations. In support of this assumption, trichinellosis had occurred in early 2000s as an outbreak in Turkey and was found to result from the consumption of minced beef illegally mixed with pork of unknown origin [18]. In Egypt, the prevalence of T. spiralis, in fresh and handled pork, was reported to range between 1.7 and 4.5% [19,20].

The reported non-significantly higher incidence of PAC among the infected women, points to a minimal effect of parasitosis on pregnancy outcomes, which is assured by the statistical analyses that excluded parasitosis as a predictor for upcoming PAC. However, statistical analyses, defined the infection by more than one type of parasites; i.e. multiple parasitosis as a highly significant predictor for upcoming PAC and this predictability parallels the number of parasites. Furthermore, statistical analyses showed that obesity and older maternal age could predict the upcoming PAC, and this ability increased by the presence of multiple parasitosis.

In line with these findings, **Lau *et al.*** [21] through a systemic review assured the relation between maternal helminthic infections and adverse pregnancy outcomes and detected multiple studies reported the safety of treatment during pregnancy with cure rates up to 90% for hookworm and Ascaris, but only 50% for Trichuris. Thereafter, **Ellwanger *et al.*** [22] out of a systemic review attributed iron deficiency anemia to nutritional factors, genetic variates and helminthic infection especially by the roundworms and hookworms and reported that iron deficiency may act as a risk factor for infections by these helminths, which aggravates the severity of anemia and advised to treat anemia to break this vicious cycle

Recently, **Kindie *et al.*** [23] in a survey study found the prevalence of anemia was moderate and was related to age, large family size, intestinal parasitosis, and pregnancy. **Khezri *et al.*** [24] also found that preterm birth was associated with maternal anemia during pregnancy. They attributed anemia to nutrient deficiencies and to parasitic infestations and recommended early detection and adequate treatment of anemia and/or its causes during pregnancy as a means to reduce the prevalence of preterm birth. A structured review of scientific literature including studies comprised populations belonged to low- and middle-income countries, mainly Africans detected gestational anemia prevalence of 40%, high prevalence of hookworms and A. lumbricoides and a positive relation between gestational anemia and these parasites, while the prevalence of malaria was not associated with the magnitude of the effect of hookworm on anemia risk during meta-regression [25].

In line with the detergent impact of intestinal parasitosis on fetal growth, an experimental study found maternal nematode infection enhanced movement of leukocytes across the neonatal blood brain barriers and alter the neuroimmune development with promotion of the Th_2/Treg environment manifested as higher expression of interleukin-4 and tissue growth factor-β family genes, pathways and integrin genes that are involved in trans-endothelial cells leucocytic transport but down-regulated the endosome vesicle formation-related genes that are essential for immunoglobulin endocytosis across the blood-brain barrier [26].

CONCLUSION

Nematode parasitosis is not uncommon among pregnant women especially those followed scheduled working hours. Single-family parasitosis per se is not a predictor, while multiple-families nematode parasitosis is a significant predictor for upcoming pregnancy-associated complications. Parasitosis in obese pregnant women especially older ones has more deleterious impact on pregnancy.

RECOMMENDATIONS

Stool analysis for detection of parasitosis early in pregnancy must be a routine to allow early detection and treatment. Parasitosis as an ecological problem requires provision of instructions to pregnant women to minimize or prevent the dependence on outdoor prepared foods and to follow strict cleansing of food

staffs before using it. Further, there must be strict laws and sanctions concerning preparations of minced meats and its related products and providers of food-quality control must provide stern observations for these factories.

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