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آموزش مهارت های کاربردی در تدوین و چاپ مقاله
Gastrointestinal Helminth Infection in Pregnancy: Disease Incidence and Hematological Alterations

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Abstract

Background: The incidence and hematological effects of helminth infection during pregnancy were investigated among pregnant women in Isiala, Mbano, Southeast Nigeria.

Methods: Totally 282 pregnant women were enlisted for the study between October 2011 and September 2012. Stool samples were examined for intestinal helminths using formalin-ether sedimentation technique. Hemoglobin (Hb) and Packed Cell Volume (PCV) levels were evaluated in venous blood samples using Sahli’s and microhaematocrit methods respectively.

Results: Forty six (16.3%) subjects were infected with at least one helminth parasite; 24 (8.5%) hookworm, 14(5.0%) and 2(0.7%) A. lumbricoides and Trichuris trichiura infections respectively. Intestinal helminthiases in pregnant women was significantly associated with age ($P<0.05$). The prevalence of intestinal helminthiases by parity was also significantly different ($P<0.05$) with primigravidae having the highest infection rate (27.5%). Hematological assessment showed that the prevalence of anemia among the women was 58.9% (mean±SD = 9.3±1.0). The differences in hemoglobin levels by age groups was statistically significant ($P<0.05$). The contributory effect of gastrointestinal helminths in anemia showed that infected pregnant women had lower mean hemoglobin (8.60±0.22g/dl) than the uninfected (9.72±0.07g/dl). Significant difference ($t$-value = 5.660, $P<0.05$) was observed between the Hb of the infected and uninfected pregnant women. In addition, infected pregnant women had mean PCV of 26.09±0.65% while the uninfected had 34.54±2.96%. The mean PCV of infected pregnant women was significantly different ($t$-value = 0.013, $P<0.05$) from that of the uninfected.

Conclusion Anti-helminthic therapy after the first trimester should be part of the antenatal programme. Intestinal helminth infection showed significant negative correlation with Hb and PCV and contributed moderately to anemia.

Keywords: Anemia, Hematology, Hookworm, Hemoglobin, Packed cell volume

Introduction

“Intestinal helminths are among the most common and widespread of human infections, contributing to poor nutritional status, anemia and impaired growth“ (1). Intestinal helminthiases are also known to aggravate pre-existing anemia by decreasing appetite and thus food and iron intake (2, 3). Worldwide, anemia is an important reproductive health problem because of its association with adverse pregnancy outcome such as increased rates of maternal and perinatal mortality, premature delivery, low birth weight, etc (4). Women in developing countries spend half of their reproductive lives pregnant and lactating and a high proportion of women in developing countries become anemic during this period. Women of reproductive age who are iron deficient but not anemic may become anemic during pregnancy as a consequence of increased iron requirements and

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expanded plasma volume. Other causes of anemia include parasitic infestations such as malaria and intestinal worms.

“Epidemiological surveys have revealed that poor sanitation and inappropriate environmental conditions coupled with indiscriminate defaecation, geophagy and contamination of water bodies are the most important predisposing factors to intestinal worm infection” (5). Practices such as hand washing, disposal of refuse, personal hygiene, wearing of shoes and others, when not done properly may contribute to the infection or picking of these worms from the environments (6).

This research investigates the prevalence of helminth infection and its hematological alterations during pregnancy. Findings of this study will serve as a tool in evidence based health education on the need to intensify efforts at preventing helminthiases and its attendant risk of anemia during pregnancy.

**Materials and Methods**

**Study Population**
Two hundred and eighty-two pregnant women between the ages of 18 - 45 years, in their various trimesters and of various parities (0 - 10) were enlisted. The women were enlisted at various ante-natal clinics (ANC). The Mbano Joint Hospital laboratory was used as the base analytical centre.

**Determination of Helminth Infection**
Fresh stool samples for helminth screening were collected from each of the 282 subjects in dry, clean, leak proof and sterilized sample containers. The samples were examined for consistency and presence of cysts, proglottids and adult worms. Concentrated saturated sodium chloride floatation and formal-ether concentration techniques were used for fecal analysis. The total number of eggs was counted under X40 magnification of a compound microscope Stool samples were processed within 8 hours of collection and examined microscopically within one hour of preparation to avoid over clearance of hookworm ova. Based on the thresholds recommended by the World Health Organization (WHO), helminth intensities were classified as light, moderate or severe (7).

**Determination of Hemoglobin Concentration**
Using a sterile syringe, 3mls of venous blood was collected from each of the subjects and transferred into a capillary tube. The specimens were centrifuged using a microhematocrit centrifuge at 3000 rpm for 5 minutes. The PCV of each specimen was determined using a Hewkley microhematocrit reader and classified as follows: mild (PCV 27-29%), moderate (PCV 19-26%), and severe (PCV below 19%). The World Health Organization (8) benchmark for anemia defined as Hb < 11g/dl was differentiated as Hb < 4g/dl- ‘very severe anemia’, Hb < 8g/dl- ‘severe anemia’, Hb < 9g/dl- ‘moderate anemia’ and Hb <11g/dl- ‘mild anemia’.

**Data Analysis**
Data entry and validation was performed in excel, and statistical analysis was done using Statistical Package for Social Sciences (SPSS) version 17.0. Values were considered statistically significant when p-values were less than 0.05 (p<0.05). Pearson Chi-square, t-test and correlations were used to determine the association between hemoglobin concentrations and helminth infection as indicators of anemia.

**Permission and Ethical Approval**
At the onset of the study, the community and household heads were well briefed on the objectives of the study. Thereafter, they were given informed consent forms to sign for their communities and households after their contents were translated to them in local languages. The study protocol was approved by the State Health authorities.

**Results**

**Helminth species and levels of infections in relation to age, trimester and parity**
The gastrointestinal helminth parasites observed in this study were hookworm (8.5%), *Ascaria*...
*lumbricoides* (5.0%) and *Trichuris trichiura* (0.7%), while mixed infection accounted for 2.1% (Table 1). Of the 282 pregnant women examined, 46(16.3%) were infected with at least one parasite species. Age specific prevalence showed that subjects of 18 - 20 years age group had the highest rate of infection (27.0%) while those of 41-45 years had the least rate (0%). The difference in infection by age groups was statistically significant (P<0.05; $\chi^2$ = 28.759, df=12). Within the trimester, pregnant women in their first trimester had the highest infection rate of 20.9% while those in their third trimester had the least (12.9%). The differences were however not statistically significant ($P$>0.05; $\chi^2$ = 6.895, df =8).

Table 1 also shows the prevalence of infection on the basis of parity. The Primigravidae had the highest prevalence (27.5%) while the Gravidae 7 group had the least rate (7.7%). Differences in the prevalence of helminth infections by parity groups was statistically significant ($P$<0.05; $\chi^2$ = 32.437, df=12).

Table 1: Prevalence of single and mixed helminth infections in relation to age, trimester and parity among pregnant women in Isiala Mbano, Nigeria

| Parameter       | Number examined | *Hookworm* n (%) | *Ascaris lumbricoides* n (%) | *Trichuris trichiura* n (%) | Mixed infection n (%) | Total n (%) | Significance |
|-----------------|-----------------|------------------|-----------------------------|---------------------------|----------------------|-------------|-------------|
| **Age (yr)**    |                 |                  |                             |                          |                      |             |             |
| 18 – 20         | 37              | 3(8.1)           | 7(18.9)                     | 0                        | 0                    | 10(27.0)    |             |
| 21 – 30         | 177             | 15(8.5)          | 5(2.8)                      | 0                        | 3(1.7)               | 23(13.0)    |             |
| 31 – 40         | 62              | 6(9.7)           | 2(3.2)                      | 2(3.2)                   | 3(4.8)               | 13(21.0)    |             |
| 41 – 45         | 6               | 0                | 0                           | 0                        | 0                    | 0           |             |
| Total           | 282             | 24(8.5)          | 14(5.0)                     | 2(0.7)                   | 6(2.1)               | 46(16.3)    | $\chi^2$=28.759, df = 12, $P$ <0.05 |
| **Trimester**   |                 |                  |                             |                          |                      |             |             |
| First           | 67              | 6(9.0)           | 5(7.5)                      | 1(1.5)                   | 2(3.0)               | 14(20.9)    |             |
| Second          | 99              | 9(9.1)           | 7(7.1)                      | 0                        | 1(1.0)               | 17(17.2)    |             |
| Third           | 116             | 9(7.8)           | 2(1.7)                      | 1(0.9)                   | 3(2.6)               | 15(12.9)    |             |
| Total           | 282             | 24(8.5)          | 14(5.0)                     | 2(0.7)                   | 6(2.1)               | 46(16.3)    | $\chi^2$=6.895, df = 8, $P$ <0.05 |
| **Parity**      |                 |                  |                             |                          |                      |             |             |
| Primigravidae   | 69              | 6(8.7)           | 12(17.4)                    | 0                        | 1(1.4)               | 19(27.5)    |             |
| Secondigravidae | 55              | 4(7.3)           | 1(1.8)                      | 0                        | 1(1.8)               | 6(10.9)     |             |
| Gravidae 3 – 6  | 145             | 13(7.0)          | 1(0.7)                      | 2(1.4)                   | 4(2.8)               | 20(13.8)    |             |
| Gravidae ≥7     | 13              | 1(7.7)           | 0                           | 0                        | 0                    | 1(7.7)      |             |
| Total           | 282             | 24(8.5)          | 14(5.0)                     | 2(0.7)                   | 6(2.1)               | 46(16.3)    | $\chi^2$=32.437, df = 12, $P$ <0.05 |

**Intensity of Infection**

The intensity of infection among pregnant women (Table 2) shows that 24(80.0%) of the pregnant women examined had single (hookworm) infection while 6 (20.0%) had mixed (hookworm and *A. lumbricoides*) infection. Fifteen (62.5%) of the women with hookworm infection had light infection while 5(20.8%) and 4(16.7%) had moderate and heavy infections respectively. Out of the preg-
nant women with mixed infection, 4 (66.7%) had light infection while 2 (33.3%) had moderate infection. Also, 14 (70.0%) of infected women had *A. lumbricoides* infection, of which 8 (57.1%) had light, 4 (28.6%) moderate and 2 (14.3%) heavy infections, respectively. Only two women were positive to *T. trichiura* infection, one of which is light while the other moderate. The differences in the intensities of infection was not statistically significant ($\chi^2 = 0.967$, df=2, $P > 0.05$).

### Table 2: Intensity of helminth infection among pregnant women in Isiala Mbano, Nigeria ($\chi^2=32.437$, df =12, $P <0.05$)

| Helminthes | Intensity classification | Intensity | n (%) single infection | n (%) mixed infection |
|------------|--------------------------|-----------|------------------------|----------------------|
|             |                          | Light     | 24 (80.0)              | 6 (20.0)             |
| Hookworm   | <2000                    | Light     | 15 (62.5)              | 4 (66.7)             |
|            | 2000 – 3999              | Moderate  | 5 (20.8)               | 2 (33.3)             |
|            | ≥4000                    | Heavy     | 4 (16.7)               | 0                    |
|            |                          | Light     | 14 (70.0)              | 6 (30.0)             |
| *A. lumbricoides* | <5000                  | Light     | 8 (57.1)               | 4 (66.7)             |
|            | 5000 – 49999             | Moderate  | 4 (28.6)               | 2 (33.3)             |
|            | ≥50000                   | Heavy     | 2 (14.3)               | 0                    |
| *T. trichiura* | <1000                  | Light     | 2 (100.0)              | 0                    |
|            | 1000 – 9999              | Moderate  | 1 (50.0)               | 0                    |
|            | ≥10000                   | Heavy     | 0                      | 0                    |

**Hemoglobin (Hb) and packed cell volume (PCV) measurement**

A total of 166 (58.9%, mean±SD = 9.3±1.0) of the pregnant women were anaemic. Out of these, 92 (32.6%) had mild anemia, 58 (20.6%) had moderate anemia while 16 (5.7%) had severe anemia (Table 3). Age specific prevalence shows that women between the ages of 31 - 40 years were most anemic with prevalence rate of 71.7%, mean±SD = 9.1±1.1) while those of 18-20 years had the least (18 (48.6%, mean±SD = 9.2±1.0) - Table 3. The differences in the hemoglobin levels of these age groups was statistically significant ($P<0.05$, $\chi^2 = 17.197$, df=9).

Table 3 also shows that the pregnant women in their second trimester (63.6% and mean±SD = 9.3±1.0) had the most severe anemia. The difference in the hemoglobin levels by trimester groups was not statistically significant ($P > 0.05$, $\chi^2 = 2.794$, df=6, mean±SD=9.3±1.0). Women in their Secondigravidae had the most severe anemia (61.8%, mean±SD = 9.3±0.9). The differences in hemoglobin levels by parity was not statistically significant ($P>0.05$, $\chi^2 = 9.034$, df=6, mean±SD = 9.3±1.0) –Table 3.

**Effect of Gastrointestinal Helminths on Anemia in Pregnancy**

The contributory effect of gastrointestinal helminths on anemia is shown in Table 4. Pregnant women who were infected with one helminth or the other were observed to have lower mean hemoglobin (Hb) of 8.60±0.22g/dl than that of the uninfected (9.72±0.07g/dl). Significant difference (T-value = 5.660, $P<0.05$) was observed between the Hb of the infected and uninfected pregnant women. In addition, pregnant women infected with one helminth or the other had a mean PCV of 26.09±0.65% while the uninfected had 34.54±2.96%. The mean PCV of infected pregnant women was also significantly different (t-value= 0.013, $P<0.05$) from that of uninfected pregnant women.
Table 3: Prevalence of anemia in relation to age, trimester and parity among pregnant women in Isiala Mbano, Nigeria

| Characteristics | Number examined | Mild <11 n (%) | Moderate<9 n (%) | Severe <8 n (%) | Total n (%) | Hemoglobin (g/dL) | Mean±SD | Significance |
|-----------------|----------------|----------------|------------------|----------------|-------------|------------------|---------|-------------|
| Age (yr)        |                |                |                  |                |             |                  |         |             |
| 18 – 20         | 37             | 7(18.9)        | 10(27.0)         | 1(2.7)         | 18(48.6)    | 9.2±1.0         |         |             |
| 21 – 30         | 177            | 63(35.6)       | 29(16.4)         | 9(5.1)         | 105(57.1)   | 9.4±0.9         |         |             |
| 31 – 40         | 62             | 21(33.9)       | 16(25.8)         | 6(10.0)        | 43(71.1)    | 9.1±1.1         |         |             |
| 41 – 45         | 6              | 1(16.7)        | 3(50.0)          | 0 (0)          | 4(66.7)     | 9.4±1.0         |         |             |
| Total           | 282            | 92(32.6)       | 58(20.6)         | 16(5.7)        | 166(58.9)   | 9.3±1.0         |         | x^2=17.197, df=9, P<0.05 |
| Trimester       |                |                |                  |                |             |                  |         |             |
| First           | 67             | 16(23.9)       | 18(26.9)         | 4(6.0)         | 38(56.7)    | 9.2±1.1         |         |             |
| Second          | 99             | 37(37.4)       | 21(21.2)         | 5(5.1)         | 63(63.6)    | 9.3±1.0         |         |             |
| Third           | 116            | 39(33.6)       | 19(16.4)         | 7(6.3)         | 65(56.0)    | 9.4±0.9         |         |             |
| Total           | 282            | 92(32.6)       | 58(20.6)         | 16(5.7)        | 166(58.9)   | 9.3±1.0         |         | x^2=2.794, df=6, P<0.05 |
| Parity          |                |                |                  |                |             |                  |         |             |
| Primigravidae   | 69             | 18(26.1)       | 15(21.7)         | 4(5.8)         | 37(53.6)    | 9.3±0.9         |         |             |
| Secondigravidae | 55             | 18(32.7)       | 15(27.3)         | 12(2.9)        | 34(61.8)    | 9.3±0.9         |         |             |
| Gravidae 3-6    | 145            | 53(36.6)       | 25(17.2)         | 11(12.4)       | 89(61.4)    | 9.3±1.0         |         |             |
| Gravidae ≥ 7    | 13             | 3(23.1)        | 3(23.1)          | 0 (0)          | 6(46.2)     | 9.4±1.0         |         |             |
| Total           | 282            | 92(32.6)       | 58(20.6)         | 16(5.7)        | 166(58.9)   | 9.3±1.0         |         | x^2=9.034, df=6, P<0.05 |

Table 4: Effect of gastrointestinal helminths on anemia among pregnant women in Isiala Mbano, Nigeria

| Pregnant women | Hb(g/dl) | PCV (%) |
|----------------|----------|---------|
| Infected       | 8.60 ±0.22** | 26.09 ±0.65** |
| Uninfected     | 9.72±0.07 | 34.54±2.29 |
| t-value        | 5.66     | 1.067   |
| P-value        | 0.00     | 0.013   |

** Significantly different at P < 0.05

Correlation between helminth infections and indicators of anemia in pregnant women

The correlation between Hb, PCV and helminth infections is shown in Table 5. Hookworm infection was observed to have a moderate highly significant negative correlation with Hb (r= -0.389, P<0.01) and PCV (r= -0.277, P<0.01). Mixed infections (Hookworm and Ascaris lumbricoides) were also observed to have a mild highly significant negative correlation with Hb (r= -0.179, P<0.01) and PCV (r= -0.192, P<0.01).

Table 5: Correlation between helminth infections and indicators of anemia among pregnant women in Isiala Mbano, Nigeria

| Helminthes    | Indicators of anemia |
|---------------|----------------------|
|               | Hb (g/dl)           | PCV (%)            |
| Hookworm      | -0.389**            | -0.277**           |
| A. lumbricoides | -0.104              | -0.069             |
| T. trichiura  | -0.056              | -0.043             |
| Mixed infection | -0.179**           | -0.192**           |

** Correlation is significant at 0.01 levels

Furthermore, A. lumbricoides and T. trichiura infections were observed to have a negative correlation with Hb and PCV respectively but were not statistically significant (P>0.05).

Discussion

The prevalence of intestinal helminth infections among the study population (16.3%) is epidemiologically significant considering the fact...
that this is an epidemiological survey involving asymptomatic subjects. It has been observed that any helminth ova or larvae present would be in very low level and possibly undetectable (9). The high prevalence of hookworm infection compared to the *A. lumbricoides* and *T. trichiura* infections may be attributed to the cultural practices of the subjects especially agriculture and also high level of unhygienic practices. This is consistent with the report of a study (10). The prevalence of parasitic infections among pregnant women differed significantly (*P*<0.05) within the age groups, indicating gestational-age dependence.

Helminth infections were also found to decrease with trimester. Findings in this study show that pregnant women in their first trimester were more infected than those in second and third trimesters. This can be attributed to the fact that treatment of helminthiases during ante natal visits is done after the first trimester. That is, pregnant women are given anthelminthic drugs after their first trimester (11). When gestational age was related to anemia, women in their second pregnancy trimester were more anemic than their counterparts in their first and third trimesters. The reason for this outcome is not apparent. However, anemia in many areas of Africa was described as usually most severe in the second trimester of gestation, especially following a period of acute infection, e.g. malaria, in the first trimester (12, 13).

This study established an association between the intensity of helminth infections and lower hemoglobin (Hb). Pregnant women with light infections were found to have low hemoglobin levels, but women with heavy infections had lower hemoglobin levels. The pathenogenicity of helminth infection shows that the disease manifests in three main phases, with the intestinal phase representing the most important period. A moderate hookworm infection according to studies will gradually produce anemia as the body reserves of iron are used up, with the severity depending on the worm load and the dietary intake of iron (12). The burden of disease imposed on helminth-infected girls and women of childbearing age, especially when pregnant, may very well define the single most important contribution of intestinal parasitic infections to the calculation of their global disease burden. This study reveals a significant difference (*P*<0.05) in the mean Hb and PCV of the infected and uninfected pregnant women. Pregnant women who were infected with at least one helminth parasite presented not just a higher frequency of anemia but also significant lower level of hemoglobin and PCV.

*Ascaris lumbricoides* and *T. trichiura* infections were also observed to have a negative correlation with Hb and PCV among infected pregnant women but were not significant (*P*>0.05). Thus, helminth infections exacerbated anemia in this setting. The WHO has suggested that anemia is of "moderate" public health importance where its prevalence is between 20% and 39.9% and "severe" if it occurs in 40% or more of the population. Given these results, the importance and potential impacts of intestinal helminthiases during pregnancy, such as anemia, are quite obvious. This indicates the need for periodical stool examinations during pregnancy as part of routine laboratory test in the prenatal control of helminthiases.

A single course of anthelmintic therapy in addition to iron-folate supplementation would significantly increase hemoglobin concentrations and improve iron status in pregnant women. As has been stated in other studies, it is necessary to modify some preventive measures of information and education and to give specific treatment before the pregnancy in order to increase some of the pregnant women's health indicators. Also, anthelmintic therapy which is inexpensive and safe during pregnancy after the first trimester should be part of the antenatal programme since malaria diagnosis and treatment is also part of the antenatal programme (14).

**Conclusion**

Pregnancy is a risk factor for intestinal helminth infection. This study established an association between the intensity of helminth infections and lower hemoglobin (Hb). There is need for periodical stool examinations during pregnancy as part of routine laboratory test in the prenatal control of intestinal helminth infection.
Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc) have been completely observed by the authors.

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کارگاه‌های آموزشی مرکز اطلاعات علمی

مقاله نویسی علوم انسانی

اصول تنظیم قراردادها

آموزش مهارت های کاربردی در تدوین و چاپ مقاله