Effects Of HIV and Intestinal Parasites Co-Infection On Hematological Parameters Among Pregnant Women Attending Selected Health Facilities In Nyeri County, Kenya.

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Summary

INTRODUCTION

Prevalence of HIV among women in Nyeri County increased from 2.5% in 2007 to 5.5 % in 2009 and 6.3% in 2013. The study aimed to determine effect of co-infection of HIV and intestinal parasites on hemogram among pregnant women attending health facilities in the County.

METHODOLOGY

A comparative cross-sectional study was conducted among 130 participants. Interview schedule was used to collect data. Stool and blood samples were processed using standard procedures. Data was analyzed using SPSS. Results: among 130 respondents 34% had intestinal protozoans infection.

RESULTS

Among 65 HIV positive respondents, 25% had *Entamoeba Coli* infection and 2% *Iodamoeba butschlii.* **Among 65 HIV negative respondents, 38% had *Entamoeba Coli,* and 6% *Iodamoeba butschli*i infection. One HIV negative respondent had *Hymenolepis nana* infection. Co-infection of HIV and intestinal parasites had significant effect on WBC ($p < 0.05$), RBC ($p < 0.05$), *Haemoglobin* ($p < 0.05$) and *haematocrit* ($p < 0.05$).**

CONCLUSION

(i) Prevalence of co-infection of intestinal protozoan parasites and HIV was high
(ii) Co-infection of HIV and intestinal protozoan parasites decreased WBC, RBC, *haemoglobin* and *haematocrit.*

RECOMMENDATION

Routine screening for intestinal parasites during antenatal healthcare and more research to verify pathogenicity of *Entamoeba Coli.*

Key words: HIV, Intestinal parasites, co-infection, pregnant women, *hemogram.*

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Introduction

Over two billion people have a chronic infection of at least one or more parasites (Neglected Tropical Diseases) which cause deaths of above five hundred thousand people per year [1,2] Women of reproductive age are among 450 million people with clinical disease due to intestinal parasite infection in developing world [3]

Pregnant women are susceptible to intestinal parasites infection due to reduced cell mediated immunity. Infection by soil-transmitted *helminthes* leads to malnutrition, anaemia, and *thrombocytopenia* due to effects of longterm inflammation, poor nutrients absorption and loss of blood [4,5]. In pregnant women, *Ascaris lumbricoides coagulopathic* properties can cause bleeding after birth [6].

In 2010, it was estimated that intestinal protozoan infections such as *cryptosporidiosis* and *amoebiais* caused more harm than the highly common soil-transmitted *helminthes*. In 2010, the burden of amoebiasis was about 2.4 Disability Adjusted Life Years (DALY) [7].

Amoebic liver abscess and amoebic colitis due to *Entamoeba histolytica* is distributed globally and is a significant health threat in virtually all nations where human faeces come into contact with water and food [8]. *Amoebiais* is estimated to kill about 100,000 people every year making it the second most deadly parasitic infection globally [8, 9].

Amoebic infection increases 1.79 times the risk of preterm delivery among infected pregnant women compared to those not infected [10].

Entamoeba histolytica can easily penetrate intestinal mucosa and barrier in the placenta of pregnant woman. The immunosuppression in pregnant woman can cause reduction in production of Immunoglobulin A (IgA) that can result to sub-chronic infection and dysfunction of the placenta that is linked to preeclampsia, premature delivery and poor fetal growth [11].

Cryptosporium spp, Microsporidia and *Giardia lamblia* infections affect human health considerably [12].

Human Immuno deficiency Virus (HIV) infection may affect the bone marrow resulting to reduced *haematopoises* and observed *cytopenias* [13]. Which means, co-infection by intestinal parasites and HIV is likely to cause increased harm [14, 15].

HIV mainly affects women, where in sub-Saharan Africa, about 1.4 million pregnant women had HIV infection in 2010. In Kenya, maternal mortality rate is high at 362 for every 100,000 live births [16, 17].

In Kenya, women are more vulnerable to HIV infection with annual need for Prevention of Mother To Child Transmission (PMTCT) at 79,000 [18]. In Rwanda, HIV positive pregnant women were infected with intestinal *helminthes* [19]. In Kenyan coast, pregnant women who were reported to have HIV, Necator americanus and *Ancyclostoma duodenale* co-infection [15].

In Nyeri County, maternal mortality rate is high at 318 per 100,000 live births and HIV infection contributes to these deaths [20]. It has been observed that HIV prevalence among women in Nyeri County has been on increase from 2.5% in 2007, 5.5% in 2009 and 6.3% in 2013 [21].

Intestinal parasites infection is an area of public health research that has been neglected in Kenya and Nyeri County in particular. Pregnant woman living with HIV is vulnerable to infection by intestinal parasites. Co-infection of HIV and intestinal parasite can lead to poor pregnancy outcomes such as low birth weight.

However, there is no epidemiological data on intestinal parasites and HIV co-infection among pregnant women in Nyeri County. The study provides data on effect of both HIV and intestinal parasites infections on haematological parameters and this data can be used as evidence for management to improve pregnancy outcomes. The study aimed to determine effect of HIV and intestinal parasites co-infection on hemogram among pregnant women.

Methodology

The study was conducted in Nyeri County, Kenya. It lies within Longitudes 36o 57' east within the equator and Latitude 0o 38' south. Nyeri County has one County referral hospital, four subcounty hospitals and twenty health centers owned by Ministry of Health. A sample of 130 (65 HIV infected, and 65 uninfected)

pregnant women was used in the study. The sample of pregnant women living with HIV was distributed proportionate to the number enrolled in selected health care facilities.

For data collection, exit interviews on socio-demographic and socio-economic characteristics were conducted among pregnant women who consented using interviewer administered questionnaires. Five militres of venous blood were collected from pregnant women and processed using Medonic M-Series 3-part *haematology* autoanalyser, Boule Medical AB. Stockholm Sweden.

Stool samples were collected in universal stool containers and processed using direct wet mount method and formol-ether concentration technique to identify intestinal parasites according to Murray [22].

The data collected was processed using SPSS software (version 20). Analysis of Variance (ANOVA) test was used to compare means and Tukey Kramer post ANOVA test was done to determine where the difference between means was.

Ethical clearance was obtained from Kenyatta University

Ethical Review Committee.

Research permit was given by National Commission for Science, Technology and Innovation. Written informed consent was sought from pregnant women before participating in the study. Privacy and confidentiality were guaranteed through out the study.

Results

The age for both groups (HIV positive and HIV negative) varied from 16 to 40 years. The age bracket 26-30 years had both the highest proportions 32% and 28% for HIV negative and HIV positive respondents respectively. Age group 36-40 years had the lowest proportion 3% among HIV negative respondents and age group 15-20 years had the lowest proportion 9% among HIV negative respondents ***(Table 1)***. Marital status varied from 73% who were married to 8% who were separated among 65 pregnant women with HIV infection. Among 65 pregnant women without HIV infection, marital status varied from 83% who were married to 5% who were separated ***(Table 1).***

Table 1: *Socio-Demographic and Socio-Economic Characteristics of Pregnant Women*

Variables	**HIV Positive**	**HIV negative**
Age	**n(%)**	**n(%)**
15 - 20 years	6(9%)	11(17%)
21 - 25 years	16(25%)	19(29%)
26 - 30 years	18(28%)	21(32%
31 – 35 years	15(23%)	12(19%)
36 – 40 years	10(15%)	2(3%)
Marital status		
Married	47 (73%)	54 (83%)
Separated	5 (8%)	3 (5%)
Single	12 (19%	8 (12%)
Level of education		
No formal education	2 (3%)	0 (0%)
Primary	19 (29%)	14 (22%)

Secondary	31 (48%)	32 (49%)
University or College	13 (20%)	19 (29%)
Occupation		
Student	1 (2%)	7 (11%)
Employed	13 (20%)	16 (25%)
Self employed	34 (52%)	23 (35%)
Unemployed	17 (26%)	19 (29%)
Income level		
Kshs 1000 and below	25 (38%)	21 (33%)
Kshs 1001- 5000	25 (38%)	23 (35%)
Kshs 5001 – 10000	13 (20%)	14 (22%)
Kshs 10001 -15000	1 (2%)	3 (5%)
Kshs 15001 – 20000	1 (2%)	1 (2%)
Kshs above 20000	0 (0%)	2 (3%)

Education level of 65 HIV positive pregnant women varied from 49% with secondary education to 3% with no formal education. Among 65 HIV negative pregnant women education level varied from 48% with secondary education to 22% with primary education ***(Table 1)***. Occupation among 65 HIV positive respondents varied from 52% self employed to 2% student while occupation among 65 HIV negative respondents varied from 35% self employed to 11% students ***(Table 1).***

Monthly income among 65 HIV positive pregnant women varied from 38% earning less than Kenya shillings (Kshs.) 1000 and 25 (38%) earning between Kshs. 1001 - 5000 to 2% earning between Kshs. 15001 - 20000 monthly. Among 65 HIV negative pregnant women, monthly income varied from 35% earning between Kshs. 1001-5000 to 2% earning between Kshs. 15001– 20000 per month ***(Table 1).***

One case of *helminthes (Hymenolepis nana)* was identified among HIV negative pregnant women from Kiganjo health center.

Among 65 HIV negative pregnant women 41.5% were infected with intestinal protozoan parasites and 58.5% were not infected while 26.2% of 65 HIV positive pregnant women were infected with intestinal protozoan parasites and 73.8% were not infected.

Thirty eight percent (38 %) of pregnant women without HIV infection and 25% of pregnant women with HIV infection were infected with *Entamoeba Coli* while 6% of pregnant women without HIV infection and 2% of pregnant women with HIV infection were infected with *Iodamoeba butschlii*

White blood cells count were 85% of pregnant women with HIV infection and 75% of pregnant women without HIV infection in the normal range to 3% of HIV infected pregnant women and 0% of pregnant women without HIV infection in the below normal range (Table 2).

Cross tabulation indicated significant difference in white blood cells between HIV infected and uninfected pregnant women where HIV positive respondents had decreased white blood cells ($\chi 2 = 5.8$, $df = 2$, $p < 0.05$)

Red blood cells count varied from 69% of pregnant women with HIV infection and 95% of pregnant women without HIV infection in the normal range to 3% of HIV infected pregnant women and 2% of pregnant women without HIV infection in above normal range ***(Table 2).***

Table 2: *Distribution Of Respondents By Haematological Parameters*

Haematological parameters	**HIV status**	
	Positive n (%)	**Negative n (%)**
WBC		
0 – 3.9 X 109 cells / L (below normal range)	2 (3%)	
4 – 11.9 X 109 cells / L (normal range)	55 (85%)	49 (75%)
12 – 15.9 X 109 cells / L (above normal)	8 (12%)	16 (25%)
Total	65 (100%)	65 (100%)
RBC		
2.49- 3.84 X 1012 cells / L(below normal range)	18 (28%)	2 (3%)
3.85 – 5.20 X 1012 cells / L(normal range)	45 (69%)	62 (95%)
5.21 – 6.55 X 10 12 cells / L (above normal)	2 (3%)	1 (2%)
Total	65 (100%)	65 (100%)
HB		
7 – 10.9 g/dl (anaemic)	22 (34%)	12 (19%)
11 – 16.9 g/dl (normal)	43 (66%)	53 (81%)
Total	65 (100%)	65 (100%)
Haematocrit		
25 – 34% (below normal)	32 (49%)	6 (9%)
35 – 49% (normal range)	33 (51%)	59 (94%)
Total	65 (100%)	65 (100%)
Platelets		
0 – 139 x 109 cells/L (below normal)	1 (2%)	1 (2%)
140 - 419 x 109 cells/L (normal)	54 (98%)	54 (98%)
Total	65 (100%)	65 (100%)

Cross tabulation indicated significant difference in red blood cells between HIV positive and HIV negative pregnant women where HIV positive pregnant women had decreased red blood cells ($\chi 2 = 15.8$, df = 2, $p < 0.001$).

Eighty one percent (81%) of pregnant without HIV infection and 66% of HIV infected pregnant women had normal haemoglobin while 19% of pregnant without HIV infection and 34% of HIV infected pregnant women were anaemic ***(Table 2)***.

Cross tabulation showed significant difference in *haemoglobin* between HIV infected pregnant women and HIV pregnant women without HIV infection where HIV infected pregnant women had decreased *haemoglobin* ($\chi 2 = 3.98$, df = 1, $p < 0.046$).

Ninety four percent of pregnant women without HIV infection and 51% of those infected had *haematocrit* (Packed Cell Volume) in the normal range while 6% of pregnant women without HIV infection and 49% of HIV infected pregnant women had *haematocrit* below normal range ***(Table 2)***. Cross tabulation indicated significant difference in *haematocrit* between HIV infected pregnant women and those not infected where pregnant women living with HIV had decreased *haematocrit* ($\chi 2 = 30.18$, df = 1, $p < 0.001$).

Ninety eight percent (98%) of HIV infected pregnant women and 98% of those without HIV infection had platelets in the normal range while 2% of HIV positive and 2% of HIV negative pregnant women had platelets below normal range ***(Table 2)***. Cross tabulation indicated no significant difference in platelets between HIV infected pregnant women and pregnant women without HIV infection ($\chi 2 = 5$, df = 1, $p > 0.05$).

Two-way-ANOVA analysis and Tukey Kramer post ANOVA test of the mean *haematological* paramaters showed that infection status (co-infection of HIV and intestinal protozoan parasites) had significant effect on white blood cells ($p < 0.05$) (Table 3), red blood cells ($p < 0.05$) (Table 3), *haemoglobin* ($p < 0.05$) (Table 3), and *haematocrit* ($p < 0.05$) (Table 3). It had no significant effect on platelets ($p > 0.05$) ***(Table 3)***.

The data was also stratified by age and age had significant effect on red blood cells alone ($p < 0.05$) ***(Table 3).*** Age of pregnant women had effect on red blood cells alone where pregnant women in age group 36-40 years old had significantly reduced red blood cells.

Table 3: Relationship Between Blood Cells and Age, HIV and Intestinal Protozoan Parasites Infections Among Respondents

		ANOVA				
SOURCE OF VARIATION	**DF**	**SS**	**MS**	**Fcalculated**	**Ftable**	**P VALUE**
HIV and intestinal parasite co-infection status	3	12.52	4.173	5.56	3.59	< 0.05
Age group	4	7.552	1.888	2.515	3.36	> 0.05
Error	11	8.258	0.7507			
Total	18	28.33				
HIV and intestinal parasite co-infection status	3	1.591	0.5302	43	3.59	<0.05
Age group	4	0.269	0.0672	5.46	3.36	<0.05
Error	11	0.1354	0.0123			
Total	18	1.995				
HIV and intestinal parasite co-infection status	3	10.108	3.369	11.619	3.59	<0.05
Age group	4	1.256	0.3141	1.083	3.36	>0.05
Error	11	3.19025	0.29002			
Total	18	14.5556				
HIV and intestinal parasite co-infection status	3	99.13	33.042	15.23	3.59	<0.05
Age group	4	10.96	2.74	1.26	3.36	>0.05
Error	11	23.8619	2.1693			
Total	18	133.9551				
HIV and intestinal parasite co-infection status	3	2016.98	672.33	0.941	3.59	>0.05
Age group	4	1617.94	404.48	0.566	3.36	>0.05
Error	11	7859.0421	714.4583			
Total	18	11,493.966				

Discussion

Majority of women were in the age group 30 years and below, married, and attained at least primary education. Most of the women were self employed and had monthly income of Kenya shillings 5,000 or below. There was high prevalence of intestinal protozoan parasites infection among pregnant women and low intestinal *helminthes* infection.

The distribution of respondents in this study by white blood cells count showed that 51.5% of the population of pregnant women in the study area had normal white blood cells count. White blood cells count was within the ranges of those described in Malawi, Tanzania and Zambia [23,24].

These results are also similar to those reported that pregnancy is associated with *leucocytosis* which begins in the second month of pregnancy through third trimester [25].

Seventy seven percent and 72% of respondents in this study had normal red blood cells, *haematocrit* and platelets count respectively. Nevertheless, it is important to note that 2% of the respondents had *thrombocytopenia*. The prevalence of *Thrombocytopenia* found in this study is low compared to 8% reported in Canada [26].

This *thrombocytopenia* can be attributed to pregnancy due to high use of platelets [. High consumption of platelets and *hemodilution* reduces platelets in pregnancy [25,27].

Twenty four per cent of pregnant women were mildly anaemic (9 – 10.9 g/dl) and 2% moderately anaemic (7 – 8.9 g/dl). The prevalence of anaemia found in this study is low compared to 56% mild anaemia and 6% moderate anaemia reported in Papua New Guniea [28].

White blood cells (WBCs) count results of pregnant women with both HIV and intestinal protozoan parasites infections compared to that of pregnant women without co-infection were significantly different ($p > 0.05$). This finding is similar to that described in Malawi, Tanzania and Zambia that HIV infection lowers white blood cells count in pregnant women. The results were similar to those reported in Nigeria [24, 29, 30].

The results of this study showed that infection of both HIV and intestinal protozoan was associated with decreased red blood cells (RBCs) among pregnant women. This finding is similar to those reported that HIV infection lowers red blood cells count in pregnant women [24].

In this study, co-infection of HIV and intestinal parasites was significantly associated with anaemia in pregnancy ($p > 0.05$). This findings were similar to that reported in Tanzania, Zambia and Malawi [24,31].

The likely cause of anaemia in these respondents is suppression of *haematopoiesis* and destruction of red blood cells. The mean maternal *haemoglobin* level in women not infected with HIV in this study was higher than values reported elsewhere for pregnant women in Africa while that of HIV infected pregnant women was lower [23].

However, the mean maternal *haemoglobin* level in HIV infected pregnant women in this study was higher than that reported in Malawi, Tanzania, Zambia and India. There was no association between intestinal *helminthes* infection and anaemia in pregnancy ($p > 0.05$). This is because only one *helminthes Hymenolepis* nana was found in this study [24].

In this study, co-infection with HIV and intestinal protozoan parasites was associated with *haematocrit* or packed cell volume in pregnancy. This finding is similar to that reported in Nigeria that *haematocrit* level is low in people infected with HIV [30,32].

The results are also similar to those reported in Bailere and in Nigeria that HIV infection in pregnant women causes reduction in packed cell volume and hence predisposes them to anaemia [33].

In this study, intestinal parasite infection alone was not associated with *haematocrit* in pregnancy ($p > 0.05$). Mean platelets count of HIV infected pregnant women and those not infected was 251 and 248 respectively. This finding is similar to that reported that HIV infection increase platelets count in pregnancy [24].

The increase was however not significantly different ($p > 0.05$). This finding differs with that reported in Nigeria[30] that HIV infection reduce platelet count during pregnancy. This difference could be attributed to

the fact that pregnant women in this study were on ART treatment and immunity had improved. Infection with intestinal protozoan parasites alone was not associated with platelet count in pregnancy. Although intestinal protozoan parasite infection alone was not significantly associated with *haematological* parameters during pregnancy in this study ($p > 0.05$).

However, co-infection of intestinal *Entamoeba Coli* and HIV was significantly associated with *haematological* parameters ($p< 0.05$) apart from platelets. This show a possibility that Entamoeba *coli* is pathogenic. This finding is similar to results reported in Turkey [34] that *Entamoeba Coli* could be pathogenic.

The strength of this study includes the study design was comparative crossectional analysis. The weakness was that the analysis of the stool samples was done using direct method and formal ether concentration technique which may not be able to clearly distinguish the parasites.

Conclusion and Recommendations

Prevalence of co-infection with HIV and intestinal protozoan parasite among pregnant women was 26%. Co-infection of HIV and intestinal protozoan parasites lowered WBC, RBC, *haematocrit* and *haemoglobin* while it had no significant effect on platelets during pregnancy. Ministry of Health Kenya should include screening for intestinal parasites a routine practice in antenatal healthcare services. More research is needed to verify *pathogenicity* of *Entamoeba coli*

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