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Magnitude of *H.pylori*infection and its association with Pre-eclampsia among pregnant women attending antenatal care at Gandhi memorial hospital Addis Ababa, Ethiopia.

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Research Project Submission Form

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Table of content

List of Table

List of Figure

Abbreviations

ALP: Alkaline Phosphatase

CagA: Cytotoxic associated antigen

CHO: Cholesterol

DREC: Department of Research and Ethics Committee.

GOT: Glutamic-oxaloacetic transaminase.

GPT: Glutamic-pyruvic transaminase

HDL: High density lipoprotein

HP: H. pylori

HPAG: *H.pylori* antigen

HPAB: *H.pylori* antibody

LDL:Low density lipoprotein

MetS: Metabolic syndrome

PE: Pre-eclamptic

TG: Triglyceride

VacA: Vacuolating cytotoxic antigen

Abstract

Background: Helicobacter pylori infections are associated with many complications of pregnancy including preeclampsia and itcolonizes the gastric mucosa of about half of the world's population. It has been suggested that *Helicobacter pylori* infection could contribute to the etio-pathogenesis of pre-eclampsia by inducing a pro-inflammatory state. The association *H. pylori* with preeclampsia needs to be further explored.

Objective: To assess magnitude of *H. pylori infection* and its association with pre-eclampticpregnant women attending antenatal care at Gandhi Memorial hospital Addis Ababa, Ethiopia, 2021.

Methods: Hospital-based case control study was conducted amongclinicallydiagnosed pre-eclamptic and Non-pre eclamptic pregnant women at Gandhi Memorial Hospital, Addis Ababa, Ethiopia. Stool samples were collected for *H. pylori*antigen test from study participants. The collected data was analyzed using statistical methods in SPSSversion23. Simple descriptive statistics was used to present the socio-demographic and clinical characteristics of the study subjects. Association between Clinical variables and *H. pylori*infection was performed with multivariate logistic regression. A p-value of <0.05 at 95% confidence level was considered as statistically significant in all the analyses.

Result:A total of 93 cases and 186 controls were included in the study giving that a response rate of 92(98.9%) and 180(96.8%) from all participants that included in the study respectively. In this study, the overall prevalence of *H. pylori*infection in all study participants was 38.9% (106/272). The prevalence of *H. pylori*infection was higher in cases than controls, 54.3% (50/92) vs. 31.1% (56/180) respectively. The mean age was 29.01(SD±4.93) years in cases and 30.37(SD± 6.2) yeas in control group. Ages 26-35 years accounted for the majority proportion for both cases 62 (67.4%) and controls 128 (71.1%). A positive association was found between *H. pylori* infection and Preeclampsia (OR: 2.64; 95% CI: 2.41–4.10).

Conclusion: *H. pylori* infection has been found to be associated with preeclampsiawomen. In this study, the prevalence of *H. pylori* infection was higher in cases than in controls (which was 54.3% vs. 31.1% respectively. "Parity, systolic blood pressure and status of hemoglobin were significantly associated with pre eclamptic women with H-pylori".**Key words***:*Helicobacter pylori, Pre-eclampsia, Pregnant women

1. Introduction

1.1 Background

*Helicobacter pylori*is a Gram- negative spiral-shaped bacterium that colonizes the gastric mucosaof about half of the world's population. It is well demonstrated that *H. pylori*induces apersisting chronic gastric inflammation in its host. Moreover, this bacterium is associated with many complications of the upper gastrointestinal tract, such as chronic gastritis, peptic ulcer and disease gastric cancer [1-4].*H. pylori* pathogenicity depends on several strain-specific factors. Some *H. pylori* strains express specific genes conferring pro-inflammatory, cytotoxic and vacuolatingproperties which could enhance thein vivo pathogenicity [2,3].

Helicobacter*pylori* infection have a role in the pathogenesis of various pregnancy-related disorders through different mechanisms: depletion of micronutrients (iron and vitamin B12) in maternal anemia and fetal neural tube defects local or systemic induction of pro-inflammatory cytokines release and oxidative stress in gastro-intestinal disorders and pre-eclampsia; cross-reaction between specific anti-*H.pylori* antibodies and antigens localized in placental tissue and endothelial cells (pre-eclampsia fetal growth restriction, miscarriage).In particular, this Gram-negative bacterium seems to be associated with hyperemesis gravid arum, a severe form of nausea and vomiting during pregnancy [2-4].During the last decade, the relationship among *H. pylori* and several extra-gastric diseases strongly emerged in literature. The correlation among *H. pylori* infection and pregnancy-related disorders was mainly focused on iron deficiency anemia, thrombocytopenia, fetal malformations, miscarriage, and pre-eclampsia [4-6].

Pre-eclampsia (PE), defined as newly diagnosed hypertension with quantified proteinuria at or after 20 weeks of pregnancy and its resolution after delivery, remains the most important cause of maternal and perinatal mortality in developed countries. Preeclampsia (PE) is characterized by endothelial dysfunction and related hypertension and coagulative disorders. It is a leading cause of perinatal and maternal morbidity and mortality [5].

Preeclampsia complicates up to 8% of all pregnancies, and it is an important direct cause ofmaternal and fetal morbidity and mortality worldwide. Thereare two different forms of PE: "maternal PE" with an exclusive maternal origin, and "placentalPE", that is characterized by

abnormal placentation and feto-placental compromise [5,6]. Despite PE has been object of intense investigation, its etio-pathogenetic mechanisms are still poorly understood. This difficulty is certainly due to the fact that PE is a syndrome where similar symptoms could origin from different pathogenic pathways. PE is characterized by a generalized vasculardysfunction and an excessive maternal inflammatoryresponse [4-7].

The exact pathogenesis of PE is not yet fully understood. However, PE was considered as an autoimmune disorder characterized by hypertension, begins with abnormal cytotrophoblast apoptosis, which leads to inflammation and an increase in the levels of anti-angiogenic factors followed by disruption of the angiogenic status. In fact, occurrence of pre-eclampsia before 30th week of pregnancy, increase the risk of its relapse by 40% [8,9].

Recent studies pointed to effective infections such as cytomegalovirus, Chlamydia,pneumonia and *H. pylori* infectionare the pathogenesis of pre-eclampsia. In terms of therelationship between*H.pylori* and pre-eclampsia, there are important pointssuch asthe prevalence of both increases with age and is closely related with the economic and social conditions of individuals.The evidences have shown that*H. pylori* with high virulence can cause inflammation and endothelial damage and also, increases platelet activityand thrombus formation in vessels [7-9].

1.2. Statement of the Problem

Low socioeconomic status and poor sanitary or hygienic conditions are associated with the prevalence of H. pylori infection [9]. Primary *H. pylori* infections occur most commonly in early childhood, with reported annual spontaneous sero reversion rates ranging from 1 to 2% both in children and adults. *H. pylori*induced chronic gastritis results in the loss of appetite, malabsorption of nutrients, and dysregulation of the gastric endocrine and growth hormone systems, all of which may contribute to childhood growth impairment [10].

Helicobacter pylori continues to represent a major global health care burden, and national reports and guidelines have aimed at developments of an optimized clinical management *H. pylori* infection is well known to be the most common human infection *worldwide [11]*.

The roles of *H. pylori* in pathogenesis more than 90 % of gastro duodenal ulcers are associated with Helicobacter pylori. This is evident from the fact that only about 10% of people infected with this organism become sick [12]. One of the major causes of mortality among pregnant women is pre-eclampsia. Pre-eclampsia (PE) is an idiopathicdisorder of pregnancy characterized byprotein uric hypertension that affects 5%-8% of all pregnancies and is associated with significant morbidity and mortality to the mother and the fetus [13].

Several risk factors have been found for the development of Metabolism syndrome (MetS), including obesity. Obesity or overweight has been identified to be extremely associated with MetS by several studies, acting as one of the most important diagnostic criteria [14, 15]. Therefore, this study aims to assess magnitude of *H.pylori* and its association with pre-eclampsia among pregnant women attending antenatal care at Gandhi memorial hospital Addis Ababa, Ethiopia that might add knowledge of health workers and researchers about *H.pylori* and pre-eclampsia association.

1.3. Significance of the study

The outcome of this study could help to the eventual build knowledge and understanding of community, health officers and practitioners about H.pylori and its association with pre-eclampsia.

The finding of this study will be used as a reference for other researchers who have interest in the area for further investigation. Itwould help the policy, health professionals and other stake holders to improve their practice with respect to handling and solving problem on factors and consequence of H.pylori and pre-eclampsia.

It would also help to implement health education programs and changes in planning any intervention in the provision of health services and health promotion in society. This study provided baseline information or data for nationwide to develop health programs for health policy and gave a strategic means of prevention for such pre-eclampsia association infection.

2. Literature review

2.1. Magnitude of*H.pylori*

Recent studies pointed to effective infections suchas cytomegalovirus, Chlamydia, pneumonia and H. pyloriin the pathogenesis of pre-eclampsia (9). In terms of the relationship between *H. pylori*and pre-eclampsia, there are important points such as: the prevalence of both increases with age and is closely related with the economic and social conditions of individuals(10).

Study done inNetherland showed that magnitude of *H. pylori* positive was46% and of them Cag A-positive was 35%. For women pregnancy complicated with PE (2.1%). *H. pylori* colonization rate in women with PE 56% compared to 46% in subjects without PE (p=0.02). CagA-positivity rate 20% in women with and 16% in women without PE (p=0.28) [13]. Another meta-analysis study indicates that 85% presented *H. pylori* at some time during the study. Of these 82% showed this microorganism in the gastric mucosa at the time of the endoscopic examination, detected by at least one of the methods used to detect the bacterium [14].

Study done inChina, Wuxi People's Hospital showed that *H. pylori* infection was 24.4% using the C13 urea breathe test, and they did not receive any specific treatment before. WC, BP, fasting BG, TG, and HDL-c of all enrolled women measured at 12±1 weeks' gestation [15].
Another study done inTwain showed that prevalence *H. pylori*seropositive were 23.6%) Maternal H. pylori seropositivity was correlated with a higher risk of developing gestational hypertension (GH) (12% vs. 1.2%, p = 0.04) [16].

Another systematic analysis study indicates that 27.8% of pre-eclampsia women and 31.5% of healthy women were sero-positive for Cag A antibodies that was not statistically significant difference. Whereas, 62.4% in the case group and 44.4% in the control group had Cag A antigen in stool samples that statistically significant difference [17]. Andcase-control study done in Iraq,at Al-Yarmouk Teaching Hospital showed that sero-positivity of IgG antibodies against Helicobacter pylori was significantly higher in pre-eclamptic women 72.5%than in control group 36% [18].

Global meta-analysis study indicates that the overall prevalence of *H. pylori* infection 45.2% but varied by geographical location from 18.2% in Apac District to 60.5% at Kawempe Health Centre. At 18.4%, Apacdistrict, (58.4%) [20]. In addition, another study done in Egypt showed that 5.54% developed preeclampsia and only 73 women satisfied the inclusion criteria, therefore they included as PE group. A control (No PE) group included 73 primigravida who did not develop hypertensive manifestations. There were non-significant ($p>0.05$) difference among women of both groups as regard the enrollment data apart from body weight and BMI that were significantly higher in women of PE versus No PE group [21].

2.2. Association of *H.pylori* with Pre-eclampsia

Several evidences suggest that subclinical infections could play a role in the onset of PE. The association between *H. pylori* seropositivity and PE was found for the first time by our group. We showed that *H. pylori*seropositivity frequency is higher in mothers with PE (51.1%) compared with women with uneventful pregnancy(11).

Recently, we found that CagA/VacA dual seropositivity is specifically associated with PE and, in particular, with "placental PE" [29]. Interestingly, Franceschi *et al* demonstrated that antibodies against the *H.pylori* virulence factor CagA cross-react *in vitro* with placental tissue reducing its invasiveness ability and it is well known that these antibodies recognize antigens localized on the surface of endothelial cells (28).

Study done Netherland showed that *H. pylori* infection significantly increased the incidence of MetS as well as other metabolic disorders, especially in pregnant women with high BMI. Multivariable logistic regression analysis showed that risk factors of MetS were high BMI and *H. pylori* infection. Besides, *H. pylori* infection increased the incidence of GDM and preeclampsia and potentially reduced the incidence of uncomplicated [13].

Study done in China, Wuxi People's Hospital showed that no significant impacts on birth weight, childhood growth and cognitive development were found to be correlated with maternal *H. pylori* seropositivity during pregnancy [16]. Inaddition, meta-analysis study showed that development and severityof PE showed positive significant correlation with BMI. Blood pressure measures and BMI showedpositive significant correlation with *H.pylori* positivity. Statistical

analysisdefined high BMI as the significant independent predictor for development of PE [17] and study done in Iraq at Al-Yarmouk Teaching Hospital showed that significant association had been observed in this study between infectionwith *H. pylori* and incidence of preeclampsia, but there was no remarkablerelationship between this infection and severity of preeclampsia [18].

Global meta-analysis study showed that H. *pylori* independently associated with enrollment using water from public wells, boreholes or springs and from rivers, lakes or streams. Urban residence and no formal education were also independently associated with *H. pylori* infection[20]. In addition, another study done in Egypt showed that there was non-significant difference among women of both groups as regard the enrollment data apart from body weight and BMI that were significantly higher in women of PE versus No PE group [21].

2.3. Conceptual framework

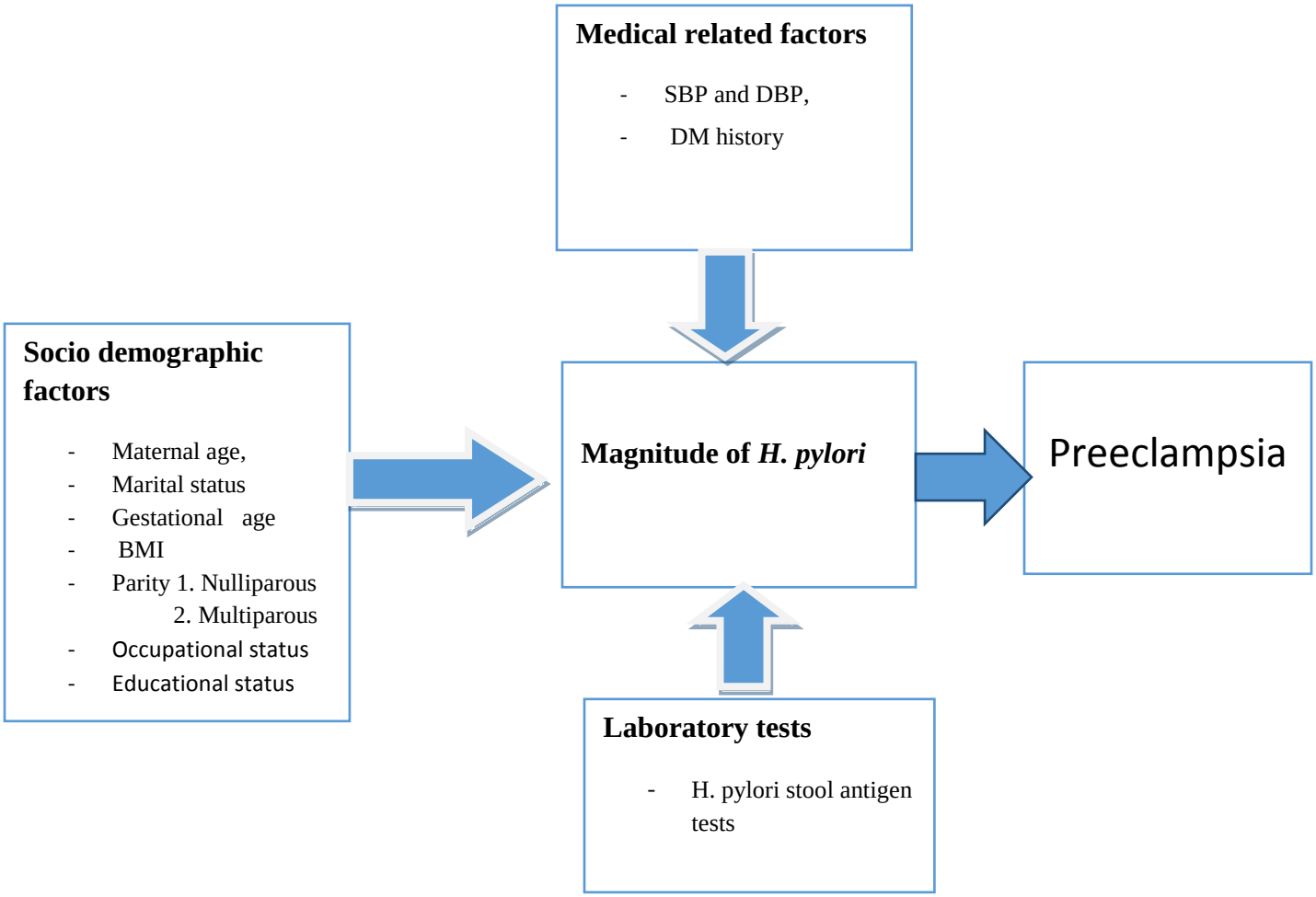

Figure 1: Conceptual framework of the study to assess magnitude of *H.pylori infection* and its association with pre-eclamptic and Non-pre eclamptic pregnant women attending antenatal care at Gandhi Memorial hospital Addis Ababa, Ethiopia, 2021[16,18]

3. Objectives

3.1 General Objective

To assess magnitude of *H.pylori*infection and its association with pre-eclamptic and Non-pre eclamptic pregnant women attending antenatal care at Gandhi Memorial hospital Addis Ababa, Ethiopia, 2021

3.2 Specific objectives

- To determine magnitude of *H.pylori*infectionamong pre-eclamptic pregnant women attending Gandhi Memorial hospital AddisAbaba, Ethiopia.
- To determine the magnitude of *H. pylori* among non pre eclamptic pregnant women's attending Gandhi memorial hospital
- To assess the associated factors of *H. pylori*infection among pre-eclamptic pregnant women on follow up at Gandhi Memorial Hospital

4. Materials and Methods

4.1 Study Area

The study was conducted in Addis Ababa, the capital city of Ethiopia, and is located in the central part of the country. Gandhi Memorial hospital serves as referral for complicated pregnancy like PE for referred cases from sub cities and country side where *H.pylori*infectionis prevalent due to life standard. The Hospital provides health services gynecology and obstetrics as well as provides other services like anti-retroviral therapy (ART), Rape clinic and NICU service family planning, voluntary counseling and testing (VCT), expanded program on immunization (EPI) and also currently new servicesopenMaternal(MICU). ANC follow-ups with maximum 36 per day and 156 per weeks in averagepregnant women attended. The hospital is under Addis Ababa city administration health bureau and is located in the central part of Addis Ababa, Kirkos sub-city in which its average service delivery is for about 100 clients per day.

4.2 Study Design and Period

A case control study design was conducted fromApril, to August 2021 at Gandhi Memorial Hospital, AddisAbaba.

4.3 Population

4.3.1 Source Population

The source population was all pregnantwomen attending antenatal care at Gandhi memorial hospital during the study period.

4.3.2 Study Population

The study population was pre-eclamptic and Non-pre eclamptic pregnancy women who attendfollow upand clinically rule out as pre-eclamptic duringstudy period and fulfilled inclusion criteria at Gandhi Memorial Hospitals.

4.4. Inclusion criteria and Exclusion criteria

4.4.1. Inclusion criteria

- All pre-eclamptic pregnant women and healthy pregnant women who was to give informed consent to participate in the study.
- Study participants who have the history and primary evaluation of SBP higher than 140 mmHg or DBP higher than 90 mmHg

4.4.2 Exclusion criteria

- ✓ Women with multiple pregnancies
- ✓ Fetal congenital malformations that could be detected by ultrasound
- ✓ Morbid obesity (BMI>40)

4.5 Study Variables

4.5.1 Dependent

- ➢ Magnitude of *H.pylori*

4.5.2 Independent

SBPand DBP, Maternal age, gestational age and body mass index before and after pregnancy, Nulliparous, Multiparous, Educational status, occupational status and diabetic mellitus.

4.6.1 Sample Size Calculation

Epi-Info version 7.2.1 was used to calculate the numberof study participant included in the study. Studyconducted in Ethiopia showed that the estimated sample sizedetermined based on the following assumptions: confidence interval of 95% at power of 80%, with the ratio of2:1 (controls to cases),proportion of*H.pylori* among pre-eclamptic pregnant mothers was 76% and proportion of *H.pylori* among Non-pre eclamptic was 57.5% (21).

$$n = \frac{\left(Z_{\alpha} + Z_{2\beta}\right)^2 \left\{p_1(1-p_1) + p_2(1-p_2)\right\}}{\left(p_1 - p_2\right)^2}$$

Where, Z_{α} = at 80% = 0.84, $Z_{2\beta}$ =at 95%= 1.96

n - size of sample in each group

P_1 - estimated population prevalence in the case groups=76%= 0.76

P_2–estimated population prevalence in the control groups= 57.5%=0.575

β = 1- Power (the probability that if the two proportions differ the test will produce a significant difference)

$$n = \frac{\left(Z_{\alpha} + Z_{2\beta}\right)^2 \left\{p_1(1-p_1) + p_2(1-p_2)\right\}}{\left(p_1 - p_2\right)^2}$$

$n= \frac{(0.84+1.96)^2 \{0.76(1-0.76) +0.575(1-0.575)\}}{(0.76-0.575)^2}$

n= 93 for each group (for the cases) and 186 for control

The final sample size was 279 (93 cases and 186controls) and there was around 98% response rate during the study.

4.6.2 Sampling Method

Convenience sampling method was used to collect the required sample sizes during the study period time.

4.6.3 Data Collection Procedure

After getting consent from the participants, small amount of stool 200mg sample was collected and processed based on each test principles for both pre-eclamptic and healthy pregnant women's.Other data like maternal age, gestational age and body mass index before and after pregnancy, history of rheumatic disease and diabetes,SBP & DBP was collected from their treatment chart.Stool samples was taken in plastic containers and sent to the laboratory as soon as possible. In case of delay in sending the samples, we have kept at 2 to 8 °C in therefrigerator for up to two weeks. The samples weremeasured for antigen rate by *H. pylori* Bosonantigen kit (Xiamen Boson Biotech diagnostic).

4.6.3.1*H.pylori*Ag testing

When an adequate volume(200 mg Stool) of extracted specimen is applied into the sample pad of the strip, the specimen migrates by capillary action across the strip. The antigens to *H. pylori* if present in the human fecal specimen was bind to the anti *H. pylori* conjugates. The immune complex is then captured on the membrane by the pre-coated *H.pylori* antigens, forming a burgundy colored 'T' band, indicating a *H. pylori* Ag positive test result. Absence of the 'T' band suggests a negative result. The test contains an internal control (C band) which should exhibit a burgundy-colored band of the immune complex of goat anti-mouse IgG/ mouse IgG-gold conjugate regardless the presence of any antigens to *H. pylori*. Otherwise, the test result is invalid and the specimen was retested with another strip[25].

4.7 Data Quality Assurance

4.6.1. Pre analytical

Stool samples were collected frompre-eclampsia pregnant women in a properly labeled cup and the tube with the patient unique identification number.

4.6.2. Analytical

The reagents and the test method were assessed with known positive and negative control materials. Based on the manufacturers' instructions, all stool samples were analyzed. Here, the standard laboratory procedures also followed and the results were checked by the laboratory quality officer and if any positive result confirmed.

4.6.3. Post analytical

The results were recorded with the unique patients' identification number and errors of test results avoided through repeatedly checking. Finally, test results of*H.pylori*submitted to Gandhi Memorial Hospitals.

4.8. Data Analysis and Interpretation

Statistical Analysis: SPSS version 23 software was used for analysis of data. Data were entered into a computer, cleaned, and analyzed using SPSS version 23 software. Descriptive statistics was performed to describe the demographic profile of the study participants. Cleaning was performed to check for frequencies, accuracy, and consistencies, and missed values and variables. Any error identified during data entry was corrected after the revision of the originally completed questionnaire.

Tables were used to present and appraise results. Bivariate and multivariate logistic regression was used to assess the association between Clinical variable’s and *H.pylori* infection. A variable with a value < 0.25 by the bivariate analysis was entered into the multivariate model. In multivariate logistic regression, a value < 0.05 was set as statistically significant for all variables.

4.9 Operational Definitions

Pre-eclampsia is a disorder of pregnancy characterized by the onset of high blood pressure and often a significant amount of protein in the urine.

HP Ag:isHelicobacter pylori (H. pylori) antigen bacteria are a common cause of peptic ulcers (sores in the lining of the stomach, small intestine, or esophagus).

Negative*H.pylori* Ag:is the status of an individual who gives a negative reaction to a serological test.

Positive*H.pylori* Ag: is the status of an individual who gives a positive reaction to a serological test.

4.10 Ethical Consideration

The study was conducted after getting the ethical clearancefrom the DepartmentResearch and Ethics Committee (DREC) of Medical Laboratory Sciences, College of Health Sciences Addis Ababa University and Ethical clearance committee of Addis Ababa city health Bureau. Official letter of request was written to Gandhi Memorial Hospital to obtain approval and carry out the study on the topic"Magnitude of *H.pylori* and its association with Pre-eclampsia among pregnant women attending antenatal care at Gandhi memorial hospital AddisAbaba Ethiopia." The Patients wasinformed about the aim of the study and assured about the confidentiality of the information and the participation was sole voluntary and none of a single service from the facility missed at the time of unwillingness or withdrawal by signing the consent form.

5. Result

5.1. Socio-demographic characteristics of study participants

A total of 93 cases and 186 controls were included in the study giving that a response rate of 92(98.9%) and 180(96.8%) from all participants that included in the study respectively**.** The mean age was 29.01(SD±4.93) years in cases and 30.37(SD± 6.2) yeas in control group. Ages 26-35 years accounted for the majority proportion for both cases 62 (67.4%) and controls 128 (71.1%). Of cases, who had primary educational status represented the majority of proportion 72(78.3%) and also in control group 112(62.2%) (Table1).

From all study subjects, 23.2% (63/272) were diabetic, of which 14.1 % (13/92) of diabetic study subjects were found in the cases group. The remaining diabetic 27.8(50/180) were found controls group**.** From all study participant's majority were multipara with 65(70.7%) of cases group and 124(68.9%) of control group (Table 1).

Table 1: Socio-demographic characteristics of pre-eclamptic and Non-pre eclamptic pregnant women attending antenatal care at Gandhi Memorial hospital Addis Ababa, Ethiopia, 2021

Characteristics		Cases n(%)	Controls n(%)
Age	18-25	27(29.3)	27(15)
	26-35	62(67.4)	128(71.1)
	> 35	3(3.2)	25(13.9)
Marital Status	Single	13(14.1)	29(16.1)
	Married	79(85.9)	151(83.9)
Educational status	Unable to read and write	3(3.2)	14(7.8)
	Primary education	72(78.3)	112(62.2)
	Secondary education	5(5.4)	39(21.7)
	College and above	12(13.1)	15(8.3)
Occupational status	House wife	53(57.6)	111(61.7)
	Private	14(15.2)	53(29.4)
	Government employee	13(14.1)	14(7.8)
	Unemployed	12(13.1)	2(1.1)
BMI	< 24	51(55.4)	120(66.7)
	≥ 24	41(44.6)	60(33.3)
Diabetes mellitus	Yes	13(14.1)	50(27.8)
	No	79(85.9)	130(72.2)
Parity	Nulliparous	27(29.3)	56(31.1)
	Multipara	65(70.7)	124(68.9)

5.2. Proportion of H-pylori among case and controls

In this study, the overall prevalence of *H. pylori*infection in all study participants was 38.9% (106/272). The prevalence of *H. pylori*infection was higher in cases than controls, 54.3% (50/92) vs. 31.1% (56/180) respectively. A positive association was found between *H. pylori* infection and PE (OR: 2.64; 95% CI: 2.41–4.10) taking non pre-eclamptic as a reference.(Table2).

Table 2: Magnitude of *H.pylori*among pre-eclamptic and Non-pre eclamptic pregnant women attending antenatal care at Gandhi Memorial hospital Addis Ababa, Ethiopia, 2021

*H. pylori*Ag	HP Ag positive	HP Ag Negative	AOR(CI)	P value
Pre-eclamptic	50(47.1%)	42(25.3%)	2.64(2.41-4.10)	0.0032
Non pre-eclamptic	56(52.9%)	124(74.7%)	1	
Total	106(100%)	166(100%)		

5.3. Association of *Helicobacter pylori* with pre-eclampsia and non-pre-eclampsia mothers

In binary logistic regression showed that body mass index, diabetes mellitus, parity, systolic blood pressure, diastolic blood pressure, status of hemoglobin and high density lipoprotein were significantly associated with pre eclamptic women with *H-pylori* at p-value less than 0.05 and 95% confidence interval. In addition, those variables significantly associated with pre eclamptic women with H-pylori at p-value less than 0.25 were candidates for multivariable logistic regression analysis (Table 3).

Multivariable logistic regression model showed that parity, systolic blood pressure and status of hemoglobin were significantly associated with pre eclamptic women with H-pylori at p-value less than 0.05 and 95% confidence interval (Table 3).

Parity of the respondent was significantly associated with pre eclamptic women with *H-pylori*. Pregnant women who were multipara were 22% decrease (AOR =0.78 (95%CI: 0.22, 0.97)) less

likely to infected with *H-pylori* as compared to their counter parts. Systolic blood pressure of the respondent was significantly associated with pre eclamptic women with *H-pylori*. Pregnant women whose systolic blood pressure were elevated were 2 times (AOR =2.1 (95%CI: 1.25, 3.88)) more likely to infected with *H-pylori* as compared to their counter parts**(Table 3)**.

Status of hemoglobin of the respondent was significantly associated with pre eclamptic women with *H-pylori.* Pregnant women who had anemia were 4 times (AOR =4.2 (95%CI: 2.1, 8.65)) more likely to infected with *H-pylori* as compared to their counter parts**(Table 3)**.

Table 3: Multiple logistic regression association of *H.pylori* with pre-eclamptic pregnant women attending antenatal care at Gandhi Memorial hospital Addis Ababa, Ethiopia, 2021

		*Helicobacter pylori*status				
Variables		Positive= n	Negative=n	COR(95% CI)	AOR(95%CI)	P - value
BMI	< 24	24	27			
	≥ 24	26	15	0.79(0.5-2.14)	0.52(0.32-1.35)	0.11
Diabetes mellitus	Yes	7	6	0.27(0.13- 0.98)	0.22(0.06-1.56)	0.2
	No	43	26	1	1	
Parity	Nulliparous	20	7	1	1	
	Multipara	30	35	0.91(0.23-1.68)	0.78(0.22-0.97)	0.03
Systolic BP	Elevated	28	18	2.4(1.1-4.64)	2.1(1.25-3.88)	0.02
	Not elevated	22	24	1	1	
Diastolic BP	Elevated	28	18	2.4(1.1-4.64)	1.92(1.34-5.32)	0.26
	Not elevated	22	24	1	1	
Hemoglobin status	Anemic	36	17	5.3(2.52-11.96)	4.2(2.1-8.65)	0.01
	Non anemic	14	25	1		
HDL	Elevated	32	16	4.8(1.65-9.24)	3.4(1.42-6.56)	0.32
	Not elevated	18	26	1	1	

In binary logistic regression showed that body mass index, diabetes mellitus, parity, status of hemoglobin and high density lipoprotein were significantly associated with non pre eclamptic women with *H-pylori* at p-value less than 0.05 and 95% confidence interval. In addition, those variables significantly associated with non pre eclamptic women with H-pylori at p-value less than 0.25 were candidates for multivariable logistic regression analysis (Table 4).

Multivariable logistic regression model showed that only high density lipoprotein was significantly associated with non pre eclamptic women with H-pylori at p-value less than 0.05 and 95% confidence interval (Table 4).

High density lipoprotein of the respondent was significantly associated with nonpre eclamptic women with H-pylori. Pregnant women whose high density lipoprotein were elevated were 20% increases (AOR =1.2 (95%CI: 1.02, 3.56)) more likely to infected with H-pylori as compared to their counter parts (Table 4).

Table 4: Multiple logistic regression association of *H .pylori* with non-pre-eclamptic pregnant women attending antenatal care at Gandhi Memorial hospital Addis Ababa, Ethiopia, 2021

		Helicobacter pylori status				
Variables		Positive N	Negative N	COR(95% CI)	AOR(95%CI)	P - value
BMI	< 24	36	84			
	≥ 24	20	40	1.2(0.8-3.2)	0.98(0.22-3.35)	0.22
Diabetes mellitus	Yes	22	28	0.23(0.05- 1.08)	0.22(0.18-1.65)	0.31
	No	34	116	1	1	
Parity	Nulliparous	18	38	1	1	
	Multipara	38	86	0.19(0.13-1.82)	0.18(0.12-1.15)	0.11
Status of hemoglobin						
	Anemic	38	94	0.67(0.42-1.36)	0.58(0.23-1.65)	0.33

	Non anemic	18	30	1		
HDL						
	Elevated	40	80	1.38(0.65-2.24)	1.2(1.02-3.56)	0.03
	Not elevated	16	44	1	1	

6. Discussion

It is estimated that *H. pylori*infection might be present in 50% of the global population. However, the pathogenic relationship between pre-eclampsiaand *H. pylori*is not well established yet because most of those infected with *H. pylori*do not complain with symptoms. In other words, the presence of *H. pylori*can be asymptomatic.Preeclampsia is a major contributor to maternal and fetal morbidity and mortality, as it complicates 2%- 8% of pregnancies. Furthermore, the problems in diagnosis of *H. pylori*infections are more complicated during pregnancy since HG could mask an active *H. pylori*infection (24). However, data concerning the association of H. pylori infection andpre-eclampsia among pregnant women obtained through case control study particularly in Ethiopia was not available. In the current study, attempt was made to investigate the association of pre-eclampsia and*H. pylori*.

In this study, the prevalence of *H. pylori* infection was higher in cases than in controls (which was 54.3% vs. 31.1% respectively and with a statistically significant level. Therefore, *H. pylori* infection has been found to be associated with pre-eclampsiawomen. It is similar with study done in Italy showed that 51.1% for case group and 31.9% for control group (24) and it also similar with study done in Iraq (25). The results in this study are relatively consistent with the study by Ponzetto *et al*. (26)The study results were also consistent with that of Cardaropoli et al. (27) , which was conducted on 111 pregnant women after dividing them into two groups: one group was the control and comprised 49 uneventful pregnancies and the other group comprised 62 women having pathological pregnancies complicated by fetal growth restriction; it was found that *H. pylori* sero-positivity was significantly more frequent in PE women with or without FGR(27).

Several studies have proposed that PE pathologic mechanism is a systemic rather than a local one. It is believed that anti-*H.pylori* IgG antibodies play a crucial role in mediating such systemic pathological effect as these immunoglobulin possess the ability to cross placental barrier (28,29).

In this study the magnitude of *H. pylori* among non-preeclamptic pregnant women was 31.1% and this was similar with many studies. High prevalence of HP among pregnant women was also reported in developing countries than developed ones [9]. Many studies conducted in different parts of the world showed the significant magnitude of HP infection: 33.3%, in Addis Ababa [22], 50.7% in Jinka [32], 19.7% in Mekelle [33], 54.7% in Halaba [34], 32.8% in Arbaminch [35], and 54.2% in Iran [36].*H. pylori* infection is quite prevalent among pregnant women (37).and is implicated in a variety of complications. It is associated with in-creased oxidative stress, as it promotes generation of reactive oxygenspecies and depletion of antioxidant molecules, such as nitric oxide and glutathione. Furthermore, CagA- producing strains are able to stimulate release of several cytokines, inducing persistent inflammatory responses. It is also hypothesized that molecular mimicry be-tween *H. pylori* antigens and host components could trigger various extra gastric manifestations via an autoimmune mechanism (37, 38).

Parity of the respondent was significantly associated with pre eclamptic women with *H-pylori*. Pregnant women who were multipara were 22% decrease (AOR =0.78 (95%CI: 0.22, 0.97)) less likely to infected with *H-pylori* as compared to their counter parts. This was similar with studies conducted in Ethiopia (12), Egypt (21) and Iraqi (25).

Systolic blood pressure of the respondent was significantly associated with pre eclamptic women with *H-pylori*. Pregnant women whose systolic blood pressure were elevated were 2 times (AOR =2.1 (95%CI: 1.25, 3.88)) more likely to infected with *H-pylori* as compared to their counter parts.This finding also supported by studies conducted in Iraqi (25), Italy (26) and Egypt (36).

In this study we observed a significant difference between HP infection and anemia; HP infection was higher in cases than control. More than two third 66(69.5%) of eclamptic women had anemia and only 40(29.1%) of non eclamptic woman had anemia. In this study, status of

hemoglobin of the respondent was significantly associated with pre eclamptic women with *H-pylori.* Pregnant women who had anemia were 4 times (AOR =4.2 (95%CI: 2.1, 8.65)) more likely to infected with *H-pylori* as compared to their counter parts. This finding was supported by studies done in Italy (26) and Egypt (36).

7. Conclusion and recommendation

7.1. Conclusion

H. pylori infection has been found to be associated with pre-eclampsiawomen. In this study, the prevalence of *H. pylori* infection was higher in cases than in controls (which was 54.3% vs. 31.1% respectively and with a statistically significant level. Parity, systolic blood pressure and status of hemoglobin were significantly associated with pre eclamptic women with H-pylori at p-value less than 0.05 and 95% confidence interval.

7.2. Recommendation

- ✓ H. pylori could be considered as one of the risk factors for pre-eclampsia in women during pregnancy. Nevertheless, several aspects need to be explored to clarify the exact role of *H. pylori* infection in pregnancies complicated with pre-eclampsia.
- ✓ Giving health education to all pregnant women to increase their awareness of H-pylori
- ✓ Further primary care level studies recommended to further explore whether screening and eradication treatment of *H. pylori*infection could benefit the mother and the fetus during the course of pregnancy.
- ✓ Finally, large- scale studies are needed to evaluate the effectiveness of *H. pylori* eradication in reducing the incidence and severity of preeclampsia

Limitation of the study

As this study was cross-sectional design, it cannot establish causality of the associations between the outcome variables and independent variables. Administering the questionnaires during a face-to-face interview may introduce social desirability bias.

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9. ANNEXES

9.1 Annex 1. Information sheet (English Version)

Research Project: Magnitude of Helicobacter pyloriinfection, pre-eclampticand Non-pre-eclamptic pregnant women at Gandhi Memorial Hospital, April to JUNE 30, 2021.

Sponsoring organization**:** Addis Ababa University, College of Health Sciences.

Principal Investigator**:** NEBIYU TSEGAYE(B.Sc. in medical laboratory science, MSc in Microbiology candidate)

Advisors: SHAMBEL ARAYA (MSc)

REGASSA DIRIBA (MSc)

Introduction

Dear participants you are kindly requested to take part in this research project as a study participant voluntarily. Read the information provided in this sheet carefully and then respond freely and voluntarily to what the investigator interviews you.

Objective of the research project:

This information sheet is prepared by the investigator and the advisors at AAU for a project with the objective Association of Helicobacter pylori infection and pre-eclampsia pregnant womenat Gandhi Memorial Hospital, January 2020.

Specific objectives: To determine the association between H. pyloriinfection and pre-eclamptic and non-preeclampsia clinical variable among pregnant women at Gandhi Memorial Hospital, January 2021.

Procedure

First of all, I would like to say thanks in advance for your cooperation and consent to participating in this study. Moreover; I requested you politely to read or listen attentively to provide relevant information about the study and if there is any confusable information or question which isn't clear regarding the study please don't hesitate to ask freely.If you are fit for the study draws 5 ml of blood samples will also be collected for only the laboratory examination of **(HPAb)** test and consent.

Benefits from participation

Study participants not yet having any financial incentives or other inducements being participated in this study. However, those positives for HP will be contacted with the physician in the hospital for treatment. Furthermore, this study provided baseline information or data for nationwide to develop health programs for health policy and Gave a strategic means of prevention for such pre-eclampsia association infection.

Risks and complication

Other than minor bleeding from the site of venipuncture when they give a sample, there are no considerable risks to the study subjects being they participate in the study. Venipuncture is a routine clinical practice for blood sample collection and can stop by pressing cotton on the site of puncture within a short period. Additionally, the amounts of blood collected were too little which is 5 ml (1 to 2 teaspoon) blood only.

Confidentiality

To maintain the confidentiality of participants' information, their name will be not given and the samples had coded. Participants were not prohibited to stop or withdraw at any time from the study. Only interested participants can retrieve their laboratory results using their code number and the information can only be accessed through the physician. The physician will be responsible for the interpretation of the results and providing treatment. No personnel information has disclosed to a third party or will be not appear in any report from this study.

9.2. Annex 2: Informed consent (English version)

I sign my signature below to confirm you that I take over the responsibility for the scientific ethical and technical conduct of the research project and for provision of progress reports for all stakeholders of the research project.

Nebiyu Tsegaye (PI)

Signature: _____ Date: _____

Note: If you have any questions about this study, you should feel free to ask now or at any time throughout the study by contacting: PI Address: Nebiyu Tsegaye: Department of Medical Laboratory Sciences, School of Health Sciences, College of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia.

E-mail:-nebiyutseg1@gmail .com Tel.: +251 9-10-09-56-29

1.2. Informed consent

I, (Full Name) ……...……

Hereby voluntarily authorize the researcher to interview me and consent to him to

obtain information that is relevant to his research topic on "Association of Helicobacter pyloriinfectionandpre-eclampsiapregnant women at Gandhi memorial Hospital Addis Ababa Ethiopia.2021 I understand that as a participant, my privacy will be maintained and the information obtained in this research will be used in a manner that protects with guaranteed confidentiality, respect, and personal rights.

I am aware that participating in this study is voluntary and I haven't obligated to answer every question asked of me and that I may withdraw my consent at any time without disadvantage to myself or others. I am informed that the information collected in this study will be strictly confidential and for this study only.

Signature of Participant …… Date……..

Signature of data collector……. Date……

Thank you!!!

9.4. Annex 4: Information sheet (Amharic version)

[illegible]

[illegible]ምንያህሉ Preeclampsia ታማሚዎች ለጨጎራ ባክቴርያ ህመም ተጋልጠዋልና መነሻ መንስሄ ነው የጨጎራ ባክቴሪያ ለ Pre-Eclampsia ተጠቂየሚለውን ለማወቅና ለተጨማሪ የጨጎራ [illegible] እ[illegible] [illegible]Pre-Eclampsia [illegible]መንስሄ[illegible] [illegible]-[illegible]

[illegible]

Title:-Association of Helicobacter Pylori infection and Pre-Eclampsia Pregnant Women at Gandhi Memorial Hospital Addis Ababa Ethiopia. Consent form for respondents in Amharic

[illegible]/[illegible]የማይክሮ ባይሎጂ ተማሪ የሆንኩት ነብዩ ፀጋዬ ስሆን ለሚሰራ ውጥናት ጥናቱ ላይ ተሳታፊ የሆኑትን ተገልጋዮች መረጃና ፍቃድ መጠየቅያ ቅፅ እርስ የጨጎራ ባክቴርያ የህመም መነሻመንስሄነውወይ ለ Pre-EclampsiaህክምጥናቱየሚካሄደውአገልግሎትእያገኙያሉታካሚዎችላይምንያህሉPre-Eclampsiaታማሚዎችለጨጎራባክቴርያህመምተጋልጠዋልናመነሻመንስሄነውወይየጨጎራባክቴሪያለ Pre-Eclampsiaተጠቂየሚለውንለማወቅናለተጨማሪባክቴሪያተጠቂእንዳይሆኑቅድመመከላከልዝግጅትናተጨባጭመነሻመረጃእንዲሆንስለሚጠቅምየታሰበጥናትነው።

እንደምንአደራችሁወይምእንደምንዋለችሁስሜ ---------------------------------

የስራድርሻለጥናቱየሚያስፈልገውንምርመራናሙናመሰብሰብነውበተጨማሪምየጥናቱተሳታፊየላቦራቶሪባለሙያነኝ። ጥናቱላይሙሉበሙሉተሳታፊየሚሆንኩትየ[illegible]የሁለተኛድግሪም microbiologyተማሪስሆንየምርምርጥናቱንየማደርገውምንያህሉPreEclampsiaታማሚዎችለጨጎራባክቴርያህመምተጋልጠዋልናመነሻመንስሄነውወይየጨጎራባክቴሪያ ለ Pre Eclampsiaተጠቂየሚለውንጥናትለማድረግነው።

ስለሆነምበጥናቱለሚሳተፉታካሚዎችበጥናቱበመሳተፎበጤናዎላይምንምአይነትጉዳትእንደማያጋጥሞእንገልፃለንበአንፃሩባለመሳተፎደሞበሆስፒታሉበሚያገኙትአገልግሎትላይምንምአይነትእክልእንደማያጋጥሞትበቅድምያእናሳውቃለን ።

በዚህምምክንያትምንምአይነትመጉላላትእንደማይደርስብኝበሚገባተረድቻለሁ፡፡ስለዚህሁኔታዉንበሚገባበማ
ጤንበፈቃደኝነትበምርምሩላይለመሳተፍለተመራማሪዉፈቃደኝነቴንሰጥቻለሁ፡፡በተጨማሪምየምሰጠዉየደም
ናሙናHPAgና፣HPABምርመራዎችብቻእንደሚዉልተነግሮኝተስማምቻለሁ፡፡ማንኛዉንምያልገባኝንነገመጠ
የቅዕድልተሰጥቶኝበሚገባቋንቋመልስአግኝቻለሁ፡፡በተጨማሪምየሁሉምየላብራቶሪምርመራዉጤቶችለተቋሞ
ችእንደሚሰጥናዉጤቴንማወቅከፈለኩማግኘትእንደምችልተነግሮኛል፡፡

እኔ_____የተባልኩ ግለሰብ ይህን ሁሉ በማገናዘብ ምርምሩ ላይ ስለኔመረጃ እና የደምናሙና ለመስጠት ተስማምቻለሁ፡፡

ፊርማ _____ ቀን _____

መረጃዉን ያስረዳዉ አካል _____ ፊርማ_____

9.7 Annex 1: Standard Operating Procedure (SOP) for blood sample collection

Sample collection, storage, and transportation.

1) Label tubes with the client's identification number (Labeling can also be done immediately after the specimen is obtained).

2) Explain the blood drawing procedure to the client and reassure her.

3) Wear rubber gloves and make the client in a comfortable position.

4) Prepare the Vacutainer tube and needle

5) Tie the tourniquet around the arm of the client just above the bend in the elbow. The

The tourniquet should be positioned 7.5cm to 10cm above the puncture site.

6) Tell the client to clench her fist

7) Using the tip of the index finger examine the phlebotomy site, feel the vein, and decide

Exactly will be to place the puncture

8) Disinfect the phlebotomy site by swabbing the skin in small outward circles with an alcohol swab or cotton wool soaked in isopropyl alcohol. Do not touch the prepared puncture site with your fingers after disinfecting the skin.

9) Insert the needle directly into the vein and withdraw peripheral blood of approximately

4 ml in the EDTA Vacutainer tube of the syringe

10) Tell the client to open his/her clenched fist

11) Release the tourniquet

12) Withdraw the needle from the vein and cover the puncture site cotton swab and hold (or have the subject hold) the pressure at the puncture site for 3 minutes or until adequate hemostasis is visible.

13) Properly discard the used materials in a safe container and tell the subject to do so if handled the cotton swabs to stop the bleeding.

14) Shipment of the samples from the collection site to the area of analysis should strictly adhered with proper packaging, labeling, and maintaining at 2-8 °C by using a cold chain.

15) Keep the samples in the refrigerator at 2-8°C or frozen at -20 °C according to the storage the time required (i.e. if the processing time is within 48 hours & longer than this time respectively).

3.2. Annex:-Safety Precautions

Adhering universal persecution for all blood born infections is better (gloves, lab coat, will be hing hands) when handling infectious materials that are referred to in the national health and safety guideline for standard safety procedure.

3.4. 3. Procedures (standard operating procedure) and principles for HPAb

Principle: -When an adequate volume of test specimen is applied into the sample pad of the strip, the specimen migrates by capillary action across the strip. The antibodies: either the IgG, the IgM, or the IgA, to H. Pylori if present in the specimen will bind to the H. Pylori conjugates. The immune complex is then captured on the membrane by the pre-coated H. Pylori antigens, forming a burgundy colored T band, indicating a H. Pylori Ab positive test result. Absence of the T band suggests a negative result. The test contains an internal control (C band) which should exhibit a burgundy colored band of the immune complex of goat anti-rabbit IgG/rabbit IgG-gold conjugate regardless the presence of any antibodies to H. Pylori. Otherwise, the test result is invalid and the specimen must be retested with another device.

Procedures:

1.Bring the specimen and test components to room temperature if refrigerated or frozen. Mix the specimen well prior to assay once thawed

2.When ready to test, open the pouch at the notch and remove the test strip. Place the strip on a clean, flat surface.

3.Fill the plastic dropper with the specimen. Holding the dropper vertically, dispense 1 drop (about 30-45 μL) of specimen into the sample pad making sure that there are no air bubbles.

Then add 1 drop (about 35 – 50 μL) of Sample Diluent immediately and wait for 15 minutes 4. Set up timer 5. Results can be read in 15 minutes.

Result Interpretation

1. **NEGATIVE RESULT:** If only the C band is developed, the test indicates that nodetectable antibodies to H. Pylori are present in the specimen. The result is negative.**2. POSITIVE RESULT:** If both C and T bands are developed, the test indicates for the presence of antibodies to H. Pylori in the specimen. The result is positive. Samples with positive results should be confirmed with alternative testing method(s) and clinical findings before a positive determination is made.**3. INVALID:** If no C band is developed, the assay is invalid regardless of color development on the T band as indicated below. Repeat the assay with a new device. Error and/or that the test reagent has deteriorated. The test should be repeated using a new strip.

3.4.2. 4. SOP FOR SCREENING HPAg TEST USING RAPIDS Tests

Principle

When an adequate volume of extracted specimen is applied into the sample pad of the strip, the specimen migrates by capillary action across the strip. The antigens to H. pylori if present in the human fecal specimen will bind to the anti H. pylori conjugates. The immune complex is then captured on the membrane by the pre-coated H.pylori antigens, forming a burgundy colored 'T' band, indicating a H. pylori Ag positive test result. Absence of the 'T' band suggests a negative result. The test contains an internal control (C band) which should exhibit a burgundy colored band of the immune complex of goat anti-mouse IgG/ mouse IgG-gold conjugate regardless the presence of any antigens to H. pylori. Otherwise, the test result is invalid and the specimen must be retested with another strip.

Procedures

1.Bring the fecal specimen, buffer and test components to RT. If refrigerated or frozen, mix the specimen well prior to assay once thawed.

2.When ready to test, open the pouch at the notch and remove the test strip. Place the strip on a clean, flat surface.

3.Shake the samples collection tube and holding the dropper vertically, dispense 3 drops of extracted specimen slowly onto the sample pad drop by drop making sure that there are no air bubbles and wait for 15 minutes.

4. Start the timer 5.Results can be read in 10 minutes.

Result Interpretation

1. **NEGATIVE RESULT:**

If only the 'C' band is developed, the test indicates that no detectable antigens to H. pylori are present in the specimen. The result is negative.

2. POSITIVE RESULT: If both 'C' and 'T' bands are developed, the test indicates for the presence of antigens to H. pylori in the specimen. The result is positive. Samples with positive results should be confirmed with alternative testing method(s) and clinical findings before a positive determination is made.

3. INVALID: If no 'C' band is developed, the assay is invalid regardless of color development on the 'T' band as indicated below. Repeat the assay with a new device. Error and/or that the test reagent has deteriorated. The test should be repeated using a new strip.

9.8 Annex 5: Standard operating procedure (SOP) for serum preparation

Aim: Effective Separation of blood products

Purpose: To standardize separating procedures so that research samples will be uniform in quality

1. Select test tube with no anticoagulant, serum separator tube (SST) and EDTA tube

2. Draw enough amount of blood (5ml) from the patient

3. Allow to stand for 20-30min for clot formation at room temperature before spinning and Separatingand also EDTA sample immediately gently mix to prevent clot formation a delay in Centrifugation may have a detrimental effect on the sample quality and may result inaccurate results. Avoid hemolysis

4. Centrifuge the sample to speed separation and affect a greater packing of cells. Clot and cells will separate from clean serum and settle to the bottom of the vessel.

NB:-The supernatant is the serum which can be now collected by dropper or pipette for testing purpose or stored (-20C to -80C) for subsequent analysis or use.

9.9. 6: Annex- Data Collection Sheet.

S/r No	Cared No	Age	Final HPAb &Ag Result		Maternal age	Gestational age	Body mass index before & after, pregnancy	history of rheumatism disease	Diabetes	SBP& DBP
			Neg	Pos						

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Declaration

I, the undersigned agree to accept responsibility for the scientific ethical and technical conduct of the research project and for provision of required progress reports as per terms and conditions of the research publications office.

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Date of submission: _____

This thesis has been submitted with our approval as advisors.

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Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.