Online Journal of Health and Allied Sciences
Peer Reviewed, Open Access, Free Online Journal
Published Quarterly : Mangalore, South India : ISSN 0972-5997
Volume 8, Issue 4; Oct - Dec 2009

Original Article:

Impact of Maternal *Helicobacter pylori* Infection on Trace Elements (Copper, Iron and Zinc) and Pregnancy Outcomes

Emmanuel Ike Ugwuja,
Department of Chemical Pathology, Faculty of Clinical Medicine, Ebonyi State University, P.M.B. 053 Abakaliki, Nigeria,
Emmanuel I Akubugwo,
Department of Biochemistry, Abia State University, Uturu, Nigeria

Address For Correspondence:
Ugwuja EI,
Department of Chemical Pathology,
Faculty of Clinical Medicine,
Ebonyi State University,
P.M.B. 053 Abakaliki, Nigeria
E-mail: ugwuja@yahoo.com

Citation: Ugwuja EI, Akubugwo EI. Impact of Maternal *Helicobacter pylori* Infection on Trace Elements (copper Iron and Zinc) and Pregnancy Outcomes. *Online J Health Allied Scs.* 2009;8(4):7
URL: http://www.ojhas.org/issue32/2009-4-7.htm
Open Access Archives: http://cogprints.org/view/subjects/OJHAS.html and http://openmed.nic.in/view/subjects/ojhas.html

Submitted: Dec 12, 2009; Accepted: Apr 2, 2010; Published: Apr 30, 2010

Abstract:
Background: *H. pylori* infection has been suggested to interfere with micronutrient metabolism and influence pregnancy outcomes. **Objectives:** This study therefore seeks to document the prevalence of *H. pylori* seroposivity among pregnant women and to determine its impact on some trace element status and pregnancy outcomes. **Materials and methods:** Three hundred and forty nine consenting pregnant women aged 15-40 years (mean; 27. 04 ± 4. 75 years) and gestational age ≤ 25 weeks (mean 21.77 ± 3.14 wks) attending antenatal clinic at Federal Medical Centre, Abakaliki, between July 2007 and September 2008 participated in the study. *H. Pylori* antibody (IgG) was determined by a new generation ELISA method. Plasma copper, iron and zinc were analysed using flame atomic absorption spectrophotometer (Bulk Scientific AVG 210 Model) while haemoglobin and albumin were analysed using standard haematological and biochemical techniques. Both maternal sociodemographic and anthropometric parameters were recorded at recruitment. The women were followed-up till delivery after which neonatal anthropometrics and other birth outcomes were recorded. **Results:** *H. pylori* seroprevalence of 24.1% (84/349) was recorded with higher prevalence in multiparous and older women. *H. pylori* infected women had significantly higher BMI (29.00 ± 3.89 vs. 26.86 ± 4.10, $p = 0.020$) and lower ($p > 0.05$) plasma levels of Cu, Fe, Zn, albumin, and haemoglobin when compared to non-infected women. Also *H. pylori* infected women had significantly ($p < 0.05$) higher rates of convulsion and concomitant illnesses than their non-infected counterparts, although there was no difference in the two groups for other pregnancy outcomes. **Conclusion:** *H. pylori* infection during pregnancy seems to interfere with trace element metabolism and contribute significantly to increased maternal morbidity. Prior to confirmation of these findings in a well controlled randomised trial, it is suggested that pregnant women be screened for *H. pylori* infection to reduce *H. pylori* associated morbidities during pregnancy.
Key Words: *H. pylori*, Pregnancy, Trace elements, Copper, Iron, Zinc, Nigeria

Introduction:
Since its discovery in 1982, *H. pylori* infection has been recognised to occur throughout the world, with seroprevalence significantly higher in developing than the developed countries.(1,2) Current evidence showed that *H. pylori* is acquired in early childhood through family members by oral-oral, gastric-oral, or oral-faecal routes and persist till adulthood.(3, 4) In Nigeria, like other developing countries, the prevalence of *H. pylori* is alarmingly high (5) and this has been attributed to poor personal and public hygiene. Infection with *Helicobacter pylori* has been associated with both gastric and extragastric clinical conditions. For example, *H. pylori* infection has been associated with gastritis, peptic ulcer, gastric adenocarcinoma, and type B low-grade mucosa associated lymphoid tissue lymphoma (6) as well as diabetes mellitus, atherosclerosis, insulin resistance, some autoimmune diseases (7-9) and iron deficiency anaemia refractory to iron supplementation.(10) Except for IDA, the role of *H. pylori* in some of these extragastric associations has been of intense debate as data have been conflicting. Recently, there has been controversy of the association between infection with *H. pylori* and intra-uterine growth restriction. For instance, while McKenna *et al.* (11) and Bromberg *et al.* (12) in Ireland and Francisco respectively, reported lack of association between *H. pylori* and outcomes of pregnancy such as preterm delivery, non reassuring foetal status during labour or both and its presence not associated with obesity or parity, Eslick and his colleagues (13) reported that intrauterine growth restriction was more common in *H. pylori*-seropositive women than in *H. pylori*-seronegative women. Deficiencies of some micronutrients such as copper, zinc manganese, vitamins A, B_{12}, C, and E have been suggested to occur concomitantly in *H. pylori* infection.(14)

Even though *H. pylori* and pregnancy have some symptoms, such as, epigastric pain/dyspepsia, flatulence, nausea, fullness, vomiting in common, studies have shown that *H. pylori* infection is neither associated with dyspeptic symptoms in pregnancy nor is it related to the severity of dyspeptic symptoms. (12,15) In *H. pylori* endemic population as evidenced in Abakaliki, pregnant women may be harbouring asymptomatic *H.pylori* infection, with its attendant deficiencies of essential micronutrients which may affect the course and outcomes of

pregnancy. This study therefore seeks to document, for the first time, the prevalence of *H. pylori* seroposivity among pregnant women and to determine the impact of *H. pylori* infection on some trace element status and pregnancy outcomes.

Materials and Methods:

This study was part of a larger study that evaluated the impact of maternal trace element (copper, iron and zinc) status on pregnancy outcomes in Abakaliki, South Eastern Nigeria. It was a longitudinal study that recruited 349 consenting pregnant women, aged 15-40 years, gestational age ≤25 weeks between July 2007 and September 2008. Gestational age was determined by date of last menstruation and confirmed by ultrasonography and where there is disagreement between the two methods that of ultrasonography was used. Exclusion criteria include suffering from chronic disease, HIV-seropositivity and multiple pregnancies. Women who were on proton pump inhibitors or on *H. pylori* eradication therapies were also excluded. The protocol for this study was approved by the Ethics and Research Committee of the Federal Medical Centre Abakaliki. The sociodemographic of the participants were collected by structured questionnaires. Maternal anthropometry; height and weight were measured with the subject in light clothes without shoes, and BMI (Kg/m2) calculated. Seven millilitres (7.0 ml) of non-fasting venous blood collected between 08.00-10.00 hours were dispensed into trace element-free heparinised plastic bottles (3.0 ml), plain bottles (2.0 ml) and EDTA bottle (2.0 ml) for analysis of biochemical, haematological parameters and for *H. pylori* serology respectively. The blood samples in the trace element-free and plain bottles were centrifuged at 2000g for five minute for the isolation of plasma and serum respectively. The plasma samples were frozen until they were analysed. Participants were followed-up weekly till delivery. At every follow-up, participants were evaluated by the attending Obstetricians for concomitant illness such as diabetes and hypertension. At delivery baby's birth outcomes such as weight, length, head circumference as well as still birth, mode of delivery, gestation age at delivery were recorded by the attending midwives. Birth weight was determined using electronic weighing balance and recorded to the nearest 0.05Kg with the scale checked periodically throughout the study for accuracy while birth length and head circumference was determined by a measuring tape to the nearest 0.1cm. Plasma copper, iron and zinc were determined in duplicates using atomic absorption spectrophotometer and the mean recorded as the absolute value. Haemoglobin concentration was determined using Cyanmethaemoglobin method as described previously.(16) Anaemia was defined as haemoglobin concentration <11.0 g/dl (17) while copper, iron and zinc deficiencies were defined plasma levels <8.0 µmol/l, < 10.0 µmol/l and < 5.0 µmol/l respectively.(18) Plasma albumin was determined as described previously (19) Determination of immunoglobulin G (IgG) to *Helicobacter pylori* was done by enzyme-linked immunosorbent assay (20) using a third generation commercial ELISA kit.

Statistical Analyses

The data collected were analysed using statistical package for social science (SPSS version 10). Percentage prevalence rates were calculated with their respective 95% confidence intervals. Differences between proportions were evaluated using the chi-square tests while differences in means were evaluated using one-way analysis of variance (ANOVA). Statistical significance were achieved at $p < 0.05$.

Results:

Table 1: Seroprevalence of *H. pylori* in relation to maternal sociodemographic and Obstetrics data (percentage in parenthesis)

Indices of SES	No Examined	*H. pylori* positive (n/%)	95% CI
Living accommodation			
Single room	189	38 (20.1)	14.4-25.8
Flat	135	40 (29.6)	21.9-37.3
Bungalow	24	5 (20.8)	0.2-41.4
Total	348	83 (23.9)	19.4-28.4
Educational level [a]			
None	8	2 (25.0)	-5.0-55.0
Primary	42	7 (16.7)	5.3-28.1
Secondary	172	34 (19.8)	13.8-25.6
Tertiary	120	40 (33.3)	25.1-41.5
Total	342	83 (24.3)	19.8-28.8
Occupation			
H/W	53	12 (22.6)	11.3-33.9
Civil servants	143	37 (25.9)	18.7-33.1
Artisans	87	21 (24.1)	15.1-33.1
Students	61	12 (19.7)	9.7-29.7
Farming	5	2 (40.0)	-2.9-82.9
Total	349	84 (24.1)	19.6-28.6
Parity			
0	140	35 (25.0)	17.8-32.2
1	66	11 (16.7)	7.6-25.8
2	53	11(20.8)	9.8-31.8
3	40	12 (30.0)	15.8-44.2
>3	50	15 (30.0)	17.3-42.7
Total	349	84 (24.1)	19.6-28.6
Age group			
< = 19	16	3 (18.8)	0-38
20-24	85	16 (18.8)	10.5-27.3
25-29	138	29 (21.3)	14.5-28.2
30-35	107	35 (32.7)	23.8-41.6
>35	5	1 (20.0)	-5.1-55.1
Total	**349**	**84 (24.1)**	**19.6-28.6**

H. pylori prevalence of 24.1% (84/349) was recorded. Although no specific trend was observed, *H. pylori* infection was higher in women who live in flat than other living accommodation (table 1). Similarly, without a particular trend, higher prevalence of *H. pylori* was observed in women who were educated up to tertiary level and women without formal education compared with women with either primary or secondary education. As for maternal occupation, highest *H. pylori* infection was found among women whose occupation was farming (2/5, 95% CI: -2.9-28.9) when compared to other occupations, with least prevalence of 12/61, 95% CI: 5.3-28.1) recorded among women who were student. Maternal *H. pylori* infection was found to increase with parity, with multiparous women having the highest prevalence while least prevalence was found among women with parity 1 (11/66, 95% CI: 7.6-25.8). However, maternal age group 30-35 years had the highest prevalence of *H. pylori* infection, with age groups < = 19 and 10-24 years having least prevalence of 18.8% each (Table 1).

Table 2: Comparison of maternal anthropometrics, haematological and biochemical parameters between *H. pylori* infected and non-infected pregnant women

Parameters	*H. pylori* seropositive (n = 84)	*H. pylori* seronegative (n = 265)	p-values
Age (yrs)	28.19 ± 4.89	26. 70 ± 4.65	0.012*
BMI (Kg/m^2)	29.00 ± 3.89	26.86 ± 4.10	0.020*
Parity	1.54 ± 1. 57	1.37 ± 1. 43	0.366
HBC (g/dl)	10.04 ± 1.22	10.27 ± 1.27	0.148
Cu (µmol/l)	8.64 ± 7.10	9.90 ± 10.03	0.285
Fe (µmol/l)	10.00 ± 7.27	10.33 ± 7.83	0.734
Zn (µmol/l)	8.79 ± 9.93	9.31 ± 8.92	0.650
Albumin (g/dl)	3.41 ± 0.82	3.47 ± 0.79	0.582
*P < 0.05 is considered statistically significant.			

From Table 2, although women who were infected with *H. pylori* were significantly older (p < 0.05), and have significantly higher BMI than non infected women, no difference was observed between the two groups in term of parity p > 0.05). Also no significant difference was found in the haemoglobin concentration (HBC) between women who were infected with *H. pylori* and those without infection, although *H. pylori* infected women had slightly higher value. For trace elements Cu, Fe, and Zn, although women who were seropositive for *H. pylori* had slightly lower values than their seronegative counterparts, these were not statistically significant (p > 0.05). The two groups also showed comparable plasma albumin concentration (p > 0.05).

Table 3: Maternal and foetal outcomes in relation to Maternal *H. pylori* serostatus (proportions; n/%)

	H. pylori serostatus		RR (95% CI)	p-values
	Negative (n = 239)	Positive (n = 79)		
Maternal morbidity				
Maternal HBC < 11.0 g/dl	188 (78.7)	64 (81.0)	1.1 (0.8-1.3)	0.47
Maternal D/M	8 (3.3)	5 (6.3)	1.9 (1.6-2.0)	0.22
Maternal hypertension	34 (14.2)	6 (7.6)	0.6 (0.4-0.7)	0.15
Convulsion[§]	1 (0.4)	3 (3.8)	9.5 (7.0-11.1)	0.02*
Concomitant illnesses[§]	147 (61.5)	63 (79.7)	2.4 (1.9-2.6)	0.00*
Pregnancy outcomes				
Instrumental delivery	24 (10.0)	5 (6.3)	0.6 (0.5-0.7)	0.24
C/S delivery	14 (5.9)	2 (2.5)	0.4 (0.3-0.6)	0.24
Preterm delivery (< 37wks)	20 (8.4)	2 (2.5)	0.3 (0.2-0.4)	0.08
LBW (< 2.5 Kg)	34 (14.2)	10 (12.7)	0.9 (0.7-1.1)	0.59
Male child	138 (57.7)	45 (57.0)	1.0 (0.8-1.2)	0.90
Still birth	11 (4.6)	1 (1.3)	0.3 (0.1-0.5)	0.18
[§](Malaria, Urinary tract infection, upper respiratory tract infection, dyspepsia); * p-values < 0.05.				

From Table 3, maternal anaemia was comparable between *H. pylori* infected and non infected women. The proportions of women with diabetes and hypertension were higher in *H. pylori* infected women than in non infected women, although not statistically significant, with an association observed between *H. pylori* infection and maternal D/M (RR 1.9 (1.6-2.0). However, both maternal convulsion and concomitant illnesses were significantly higher in women with *H. pylori* infection when compared to their non infected counterparts. Additionally, strong positive associations were observed between the two co-morbid conditions and *H. pylori* (RR: 9.5 and 2.4 for convulsion and concomitant illnesses respectively). Although all the pregnancy outcomes (instrumental delivery, C/S, preterm delivery, LBW, male sex and still births) were lower in women infected with *H. pylori* than non infected women, these were not up to statistical significant level. However, while no association was found between male sex and *H. pylori*, all other outcomes showed negative associations.

Discussion:

This study has documented *H. pylori* seroprevalence of 24.1% among pregnant women which was associated with poor socioeconomic status. It also showed that *H. pylori* infection during pregnancy is associated with increased BMI, convulsion and concomitant illness such as malaria, urinary tract infections, upper respiratory tract infections and dyspeptic symptoms. *H. pylori* prevalence of 24.1% is comparable to the value obtained in an earlier study (26.3%) of uninvestigated dyspeptic individuals in the same population. It is also comparable to *H. pylori* prevalence of 20% reported by Eslick et al. (13) among pregnant women in Australia. However, it is lower than 41.8% and 65.7% reported among pregnant women in Northern Ireland and Francisco respectively.(11,12) The lower seroprevalence of *H. pylori* in the present study may be due to study populations. While theirs were done on pregnant women with dyspeptic symptoms specifically, the present study was conducted among pregnant women generally, irrespective of dyspeptic complaint. Data have been however conflicting on the association between *H. pylori* infection and dyspeptic symptoms.(5,12,15) The higher prevalence of *H.*

pylori seropositivity in women without formal education and women who were educated to tertiary level represents two extreme cases. For the former, it could be attributable to poor personal hygiene associated with low socioeconomic status. Poor personal hygiene has been shown to enhance the acquisition and the spread of the bacteria.(5,21) These socioeconomic factors may also have accounted for the higher prevalence of the bacteria in multiparous women and in women whose occupation was farming in comparison to nulliparous or other occupational groups. However, in the latter, it could be due to persistency of earlier acquired infection during childhood (22) as evidenced by higher seroprevalence in older age groups in the present study. In the present study, *H. pylori* infection was not found to be associated with maternal parity in corroboration with the findings of Bromberg et al.(12) However the higher BMI in women infected with *H. pylori* when compared with women who were not infected with the bacteria contrasts earlier findings.(12) In an earlier study in this population, higher BMI and dyslipidaemia was associated with *H. pylori* infection (23), a phenomenon that has been speculated to be due to enhanced response to insulin leading to reduced plasma glucose levels (24) and consequent alteration in lipid metabolism. The lack of significant effects of *H. pylori* infection on maternal haemoglobin concentration (HBC) in the present study is in contrast with previous findings.(10,25-27) Although the reason for this disparity is obscure, it may be due to the effect of bacterial load, as only *H. pylori* infection with low bacterial load was associated with lower maternal haemoglobin concentration.(25) However, this study did not assess *H. pylori* bacterial load. Difference in subjects may be another reason. With the exception of the study by Farag and colleagues (25), other studies were carried out on non pregnant subjects.(10,27) The non-significantly ($p > 0.05$) lower concentrations of Cu, Fe and Zn in *H. pylori*-infected when compared with non-infected women indicate that *H. pylori* infection may have effect on trace element metabolism. Data on the impact of *H. pylori* infection on trace element status in pregnant women is scarce but few studies have shown that *H. pylori* affects iron metabolism.(10,28) Possible mechanism by which *H. pylori* affects iron metabolism include (1) decreased absorption from hypo-or achlorhydria resulting from chronic gastritis (29), (2) decreased gastric juice ascorbic acid concentration which is known to facilitate iron absorption by reduction of iron III to iron II (30), (3) increased hepcidine production associated with *H. pylori* gastritis (31), (4) uptake of iron by *H. pylori* for growth (32), and (5) decreased availability of iron by sequestration of iron in lactoferin in the gastric mucosa through a receptor-mediated process.(33) In addition to iron, a gene, copA, associated with copper transport, has been isolated from *H. pylori* strains. Again, copper metalloenzymes, superoxide dismutase involved in scavenging reactive oxygen molecules (ROMs) have been found to be affected by *H. pylori* infection.(34) Although, the exact mechanism by which *H. pylori* affect copper and zinc nutriture during pregnancy is yet to be elucidated, it may be through its effect on BMI as studies have shown that plasma zinc decreases with higher maternal BMI. Zinc metabolism, transport and/or tissue distribution has been suggested to be related to BMI through a yet-to be identified mechanism.(35) However, it has been established that plasma volume correlates significantly with BMI. (36) Thus, the lower plasma copper and zinc concentration observed in *H. pylori* infected pregnant women when compared to non-infected women in the present study may be attributable in part, to higher plasma expansion in the former than in the latter as evidenced by non-significantly lower plasma albumin in *H. pylori* infected women. However, the extent by which *H. pylori* affect plasma volume expansion is yet unknown. This study shows that *H. pylori* infection during pregnancy is associated with increased risk of maternal convulsion and concomitant illnesses, such as malaria, dyspepsia, UTI and URTI. This is in contrast with the findings of Bromberg et al. (12) and Mckenna et al.(11) However, recent meta-analyses indicated that eradication of *H. pylori* is effective in resolving symptoms in *H. pylori* positive non-ulcer dyspepsia.(37,38) The lack of association between *H. pylori* infection and pregnancy outcomes as recorded in the present study is corroboration with some earlier studies (11,12) but contrast the findings of Eslick et al. (13) where *H. pylori* seropositivity was found to be an independent risk factor for intrauterine growth restriction (OR=2.59; 95% CI, 1.12–5.95; *P*=0.025). Our patients were from *H. pylori* endemic population and must have had long tern adjustment which probably may have accounted for the lack of effect on pregnancy outcomes. It seems *H. pylori* infection during pregnancy may interfere with trace element metabolism and contribute significantly to increased maternal morbidity. This findings need to be confirmed in a well controlled randomised trial. However, it is suggested that *H. pylori* screening be part of antenatal care to reduce maternal morbidities associated with *H. pylori* infection during pregnancy.

References:

1. Pounder RE, Ng D. The prevalence of *Helicobacter pylori* infection in different countries. *Aliment Pharmacol Ther* 1995;9:33-39.
2. Taylor DN, Parsonnet J. Epidemiology and natural history of *Helicobacter pylori* infection. In: Blaser MJ, Smith PD, Ravin JI, Greeberg HB, Guerrant RL, eds. Infections of the gastrointestinal tract. New York: Raven Press, 1995: 551-63.
3. Thomas JE, Dale A, Harding MW, Coward WA, Cole TJ, Weaver LT. *Helicobacter pylori* colonization in early life. *Paediatr res* 1999;45:218-23
4. Parsonnet J, shmuely H, Haggerty T. Faecal and oral shedding of *Helicobacter pylori* from healthy infected adults. J*AMA* 1999;282:2240-5
5. Ugwuja EI, Ugwu NC. *Helicobacter pylori* in uninvestigated dyspepsia in primary health cares in Abakaliki, Nigeria. *Online J Health Allied Sci* 2007;1: 4
6. Naito Y, Yoshikawa T. Molecular and cellular mechanisms involved in *Helicobacter pylori*-induced inflammation and oxidative stress. *Free Radic Biol Med* 2002 33:323-336.
7. Leontiadis GI, Sharma VK, Howden CW. Non-gastrointestinal tract associations of Helicobacter pylori infection. *Arch Intern Med* 1999;159:925-40.
8. Aslan M, Horoz M, Nazligul Y et al. Insulin resistance in *H. pylori* infection and its association with oxidative stress. *World J gastroenterol* 2006;12(42):6865-6868.
9. Gulcelik NE, Kava E, Demirbas B, Culha C, Koc G, Ozkava M, Cakal E, Serter R, Aral Y. *Helicobacter pylori* prevalence in diabetic patients and its relationship with dyspepsia and autonomic neuropathy. *J Endocrinol Invest* 2005;28(3):214-7.
10. Chen LH, Luo HS. Effects of *H. pylori* therapy on erythrocytic and iron parameters in iron deficiency anaemia patients with *H. pylori*-positive chronic gastritis. *World J Gastroenterol* 2007;13(40):5380-5383.
11. Mckenna D, Waston P, Dornan J. *Helicobacter pylori* infection and dyspepsia in pregnancy. *Obstet Gynaecol* 2003;102(4):845-9.
12. Bromberg SH, Takei K, Garcia SA, Vitor Ada C, Zanoto A, Baracat FF. *Helicobacter pylori* infection and its correlation with gastrointestinal symp-

toms and outcome of pregnancy. *Rev Assoc Med Bras* 2006;52(5):318-22.
13. Eslick GD, Yan P, Xia H-X H, Murray H, Spurrett B, Talley NJ. Foetal intrauterine growth restrictions with *Helicobacter pylori* infection. *Alimen Pharmacol Ther* 2002;16(9):1677-1682
14. Yakoob J, Jafri W, Abid S. Helicobacter pylori infection and micronutrient deficiencies. *World J Gastroenterol* 2003;9(10):2137-2139.
15. Tanriverdi HA, Ustundag Y, Tekin IO, Barut A. Dyspeptic complaints after 20 weeks of gestation are not related to *Helicobacter pylori* seropositivity. *Med Sci Monit* 2005;11(9):CR 445-8.
16. Dacie JV, Lewis SM. *Practical Haematology*. VIII edn. Edinburgh: Churchill Livingstone 1994.
17. World Health Organisation. The prevalence of anaemia in women: a tabulation of available information. W. H. O., Geneva, 1992.
18. Robets WL, McMillin GA, Burtis CA, Bruns DE. In: Reference information for Clinical Laboratory. Tietz textbook of Clinical Chemistry and Molecular Diagnosis (ed) Burtis, C. A., Ashwood, E. R. and Bruns, D. E., 4th edition, Saunders Elsevier, St. Louis Missouri, India 2006. Pp.2251-2302.
19. Reinhold JG. Total protein, albumin and globulin. In M. Reiner (ed). Standard methods in clinical chemistry Vol. Academic Press, New York. 1953. pp: 88.
20. Pronovost AD, Rose SL, Pawlak JW et al. Evaluation of new immunodiagnostic assay for *Helicobacter pylori* antibody detection: correlation with histopathological and microbiological results. *Journal of Clinical Microbiology* 1994;32:46-50
21. Woodward M, Morrison C, McColl K. An investigation into factors associated with *Helicobacter pylori* infection. *J Clin Epidemiol* 2000;53:175-181.
22. Kivi M, Tindberg Y. *Helicobacter pylori* occurrence and transmission: a family affair? *Scand J Infect Dis* 2006;38(6-7):407-17.
23. Ugwuja EI, Ugwu NC. Plasma lipids in *H. pylori* infected pregnant women. *International Journal of Medicine and Medical Sciences* 2009;1(5):224-226.
24. Ioannis DK, Ioannis S, Panagiotis D, Vasilios G, Konstantinos D, Aggeliki D Impact of body mass index on fasting blood glucose concentration among *Helicobacter pylori* carriers. *Dig. Dis.* 2003;21:262-265.
25. Farag TH, Stoltzfus RJ, Khalfan SS, Tielsch JM. *Helicobacter pylori* infection is associated with severe anaemia of pregnancy on Pemba Island, Zanzibar. *Am j Trop Med Hyg* 2007;76(3):541-548.
26. Kearney DJ. *Helicobacter pylori* infection and iron deficiency anaemia: accumulating evidence in support of a real association. *Indian Journal of Gastroenterology* (Editorial) 2005;24:147-150.
27. Rafeey M, Zohdy S, Farhang S. *H. pylori* infection and iron deficiency anaemia in children. *Res J Biol Sci* 2008;3(6):669-672.
28. Cardenas VM, Mulla ZD, Ortiz M, Graham DY. Iron deficiency and *Helicobacter pylori* infection in the United States. *Am J Epedemiol* 2006;163:127-134.
29. Lombard M, Chua E, O'Toole P. Regulation of intestinal non-haem iron absorption. *Gut* 1997;40:435-9.
30. Charlton RW, Bothwell TH. Iron absorption. *Annu Rev Med* 1983;34:55-68.
31. Pellicano R, Rizzetto M. Is hepcidin the bridge linking *Helicobacter pylori* and anaemia of chronic infection? A research proposal *Panminerva Medica* 2004;46:165-9.
32. Doig P, Austin JW, Trust TJ. The *Helicobacter pylori* 19.6-kilodalton protein is an iron-containing protein resembling ferritin. *J Bacteriol* 1993;61:2694-7.
33. Dhaenens L, Szczebara F, Husson MO. Identification, characterization, and immunogenicity of the lactoferin-binding protein from *Helocobacter pylori*. *Infect Immun* 1997;65:514-8.
34. Gotz JM, Thio JL, Verspaget HW, Offerhaus GJ, Biemond I, Lamers CB, Veenendaal RA. Treatment of *Helicobacter pylori* infection favourably affects gastric mucosal superoxide dismutases. *Gut* 1997;40:591-596.
35. Marreiro DDN, Fisberg M, Cozzolino SMF. Zinc nutritional status in obese children and adolescents. *Biol Trace Elem Res* 2002;86:107-122.
36. Pearson TC, Guthrie DL, Simpson J, Chinn S, Barosi G, Ferrant A, Lewis SM, Najean Y. Interpretation of measured red cell mass and plasma volume in adults: expert panel on radionuclides of the International Council for Standardization in Haematology. *Br J Haematol* 1995;89:748-756.
37. Moayyedi p, Soo S, Deeks J et al. on behalf of the Dyspepsia Review Group. Systemic review and economic evaluation of *Helicobacter pylori* eradication treatment for non-ulcer dyspepsia. *BMJ* 2000;321:659-664.
38. Blum AL, Tally NJ, O'Morain C et al. Lack of effect of treating *Helicobacter pylori* infection in patients with non-ulcer dyspepsia. *N Engl J Med* 1998;339:1875-1881.