The Effect of Helicobacter pylori Infection on Prolactin Levels in Child-bearing Females at El-Ingaz Medical Center, Khartoum State-Sudan
Ahmed O. Abd Algader Ahmed1, Abubakr H. Attaalmolaa2, Albadawi A. Talha3, Youssif A. Elhameed4, Mohammed M. Saeed5, Mosab N. M. Hamad6
1Department of Medical Laboratory and blood bank, National Cancer Institute, Gezira University-Sudan
2Department of Clinical Chemistry, College of Medical Laboratory Sciences, Gezira University-Sudan
3Department of Parasitology, College of Medical Laboratory Sciences, Gezira University-Sudan
4Department of Medical Laboratory and blood bank, National Cancer Institute, Gezira University-Sudan
5Laboratory director, Al Motakamil Medical Center, Khartoum –Sudan
6Department of Medical Laboratory Sciences, Elsheikh Abdallah Elbadri University, Sudan

INTRODUCTION
Helicobacter pylori infection is well known to be the most common human infection worldwide on the basis of the fact that approximately 50% of the worldwide populations are infected and that human beings are the main reservoir [1, 2].World health organization (WHO) estimates indicate high infection rates among the world populations, most infected subjects develop no clinical symptoms or peptic ulceration and continue their life with superficial chronic gastritis [3-6]. However, approximately 17% of the infected subjects will develop peptic ulcers and one quarter of such patients (approximately 4.25%) even experience ulcer complications [7] and 1% will progress to gastric cancers [7, 8]. Helicobacter pylori, previously Campylobacter pylori, are gram-negative, microaerophilic bacterium found usually in the stomach. It was identified in 1982 by Australian scientists Barry Marshall and Robin Warren, who found that it was present in a person with chronic gastritis and gastric ulcers, conditions not previously believed to have a microbial cause. It is also linked to the development of duodenal ulcers and stomach cancer. However, over 80% of individuals infected with the bacterium are asymptomatic, and it may play an important role in the natural stomach ecology [2]. More than 50% of the world’s population harbor H. pylori in their upper gastrointestinal tract. Infection is more prevalent in developing countries, and incidence is decreasing in Western countries. H. pylori is helical shape (from which the genus name is derived) is thought to have evolved to penetrate the mucoid lining of the stomach [9]. H. pylori are found in the mucus, on the inner surface of the epithelium, and occasionally

Abstract: H pylori infection is well known to be the most common human infection worldwide on the basis of the fact that approximately 50% of the worldwide populations are infected and that human beings are the main reservoir. Infection is more prevalent in developing countries, and incidence is decreasing in Western countries. Moreover, it has been proved to be associated to endocrine system regulation of various hormones in the body, prolactin (PRL) is one of the reproductive hormones, which is secreted by the anterior pituitary gland. The biological actions of prolactin hormone are Lactotrophic effects, metabolic effects influence to action of other hormones, osmoregulatory effects, and Influence of prolactin on the immunological system. This study was conducted in Khartoum State at the period from December 2015 to May 2016, and aimed to determine the possible effect of H. pylori infection on serum prolactin levels in Child-bearing females age (15 – 45). Sample size was 50 females infected with H. pylori and 50 uninfected females as control group. Stool Samples were taken from the entire study subject (100) samples to confirm the results of H. pylori Ab ICT in serum. The stool samples were analyzed by using H.pylori stool Ags detections ICT. Serum prolactin level was measured by using Cobas e411 chemistry analyzer, the results showed that Prolactin concentration was higher in the patients compared with controls. In this study hyperprolactinemia due to H.pylori infection was reported in 52% of the patients. Statistical analysis showed that there was significant difference in the mean of prolactin concentration between infected and uninfected females group (P= 0.000). Regarding correlation between prolactin concentration and subject’s age, H. pylori infection duration or recurrence of infection, no significant was found to be observed.

Keywords: H pylori, Prolactin, Ags, hyperprolactinemia, child-bearing females.

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inside the epithelial cells themselves [10]. It adheres to the epithelial cells by producing adhesions, which bind to lipids and carbohydrates in the epithelial cell membrane. One such adhesion, Bab A, binds to the Lewis b antigen displayed on the surface of stomach epithelial cells [11]. Another such adhesion, Sab A, binds to increased levels of sialyl-Lewis x antigen expressed on gastric mucosa [12].

In addition to using chemotaxis to avoid areas of low pH, _H. pylori_ also neutralizes the acid in its environment by producing large amounts of urease, which breaks down the urea present in the stomach to carbon dioxide and ammonia. The ammonia, which is basic, then neutralizes stomach acid [13]. The pathogenesis of _H. pylori_ depends on its ability to survive in the harsh gastric environment characterized by acidity, peristalsis, and attack by phagocytes accompanied by release of reactive oxygen species [14]. In particular, _H. pylori_ elicit an oxidative stress response during host colonization. This oxidative stress response induces potentially lethal and mutagenic oxidative DNA adducts in the _H. pylori_ genome [15].

Surviving the DNA damage induced by oxidative stress appears to be supported by transformation-mediated recombinational repair. Thus, transformation and recombinational repair appear to contribute to successful infection [14]. Colonization with _H. pylori_ is not a disease in and of itself, but a condition associated with a number of disorders of the upper gastrointestinal tract. Testing for _H. pylori_ is recommended if peptic ulcer disease or low-grade gastric MALT lymphoma is present, after endoscopic resection of early gastric cancer, first-degree relatives the gastric cancer, and in certain cases of dyspepsia, not routinely [16]. Several ways of testing exist. One can test noninvasively for _H. pylori_ infection with a bloodantibody test, stool antigen test, or with the carbon urea breath test (in which the patient drinks $^{13}$C—or $^{15}$C-labelled urea, which the bacterium metabolizes, producing labelled carbon dioxide that can be detected in the breath). Also, a urine ELISA test with 96% sensitivity and 79% specificity is available. Though it is almost special cases that bacterial species produce glucosyl sterols, plants and fungi universally produce various glucosyl sterols such as glucosylsitossteroland glucosyl [12].

Prolactin is structurally related to GH and human placental lactogen. Considered a stress hormone, it has vital functions in relationship to reproduction. Prolactin is classified as a direct effector hormone (as opposed to a tropic hormone) because it has diffuse target tissue and lacks a single endocrine end organ. Prolactin is unique among the anterior pituitary hormones because its major mode of hypothalamic regulation is tonic inhibition rather than intermittent stimulation. Prolactin inhibitory factor (PIF) was once considered a polypeptide hormone capable of inhibiting prolactin secretion; dopamine, however, is the only neuroendocrine signal that inhibits prolactin and is now considered to be the elusive PIF. Any compound that affects dopaminergic activity in the median eminence of the hypothalamus will also alter prolactin secretion. Examples of medications that cause hyperprolactinemia include phenothiazines, butyrophenones, metoclopramide, reserpine, tricyclic antidepressants, _-metyldopa, and antipsychotics that antagonize the dopamine D2 receptor. Any disruption of the pituitary stalk (e.g., tumors, trauma, or inflammation) causes an elevation in prolactin as a result of interruption of the flow of dopamine from the hypothalamus to the lactotropes, the pituitary prolactin-secreting cells. TRH directly stimulates prolactin secretion, and increases in TRH (as seen in primary hypothyroidism) elevate prolactin levels. Estrogens also directly stimulate lactotropes to synthesize prolactin. Pathologic stimulation of the neural sucking reflex is the likely explanation of hyperprolactinemia associated with chest wall injuries. Hyperprolactinemia may also be seen in renal failure and polycystic ovary syndrome.

Physiologic stressors, such as exercise and seizures, also elevate prolactin. The feedback effecter for prolactin is unknown. Although the primary regulation of prolactin secretions is tonic inhibition (e.g., dopamine), it is also regulated by several hormones, including gonadotropin releasing hormone, thyrotropin-releasing hormone and vasoactive intestinal polypeptide. Stimulation of breasts, as in nursing, causes the release of prolactin secreting hormones from the hypothalamus through a spinal reflex act. As mentioned, the physiologic effect of prolactin is lactation. The usual consequence of prolactin excess is hypogonadism, either by suppression of gonadotropin secretion from the pituitary or by inhibition of gonadotropin action at the gonad. The suppression of ovulation seen in lactating postpartum mothers is related to this phenomenon [17].

**MATERIALS AND METHODS**

**Study design:**

Case control study

**Study area:**

This study was conducted in Khartoum state

**Sample size:**

100 participants, 50 were study group and 50 were control group

**Study population**

Women attending Ingaz Medical Center. Women at child bearing age (15–45) years, and whom voluntarily accepted to enroll were included in the study, women out of reproductive age (15–45) years, taking contraceptive drugs or had any reproductive problem were excluded from the study, 100 samples were selected from 130 subjects; 50 subjects and 50 controls. Then blood samples were collected to measure serum prolactin level at both groups.

DATA COLLECTION
Data was collected using questionnaire. Questionnaires were filled by the investigator.

DATA ANALYSIS
Data was analyzed and tabulated using statistical package for social sciences (SPSS) program version 20 to utilize the results.

Ethical consideration
Ethical consent was approved and signed for the implication of this study by all study participants.

Laboratory methods
Sample collection
Three ml of venous blood was collected from the participants by using a sterile needle and syringe into a labeled plain container. Clotted blood sample was then centrifuged to obtain the sera. All sera were kept at −20°C until using for measurement of prolactin hormone levels, then feces specimen was collected in clean, dry, sterile container.

Serum prolactin
First incubation: 10 μL of sample and a biotinylated monoclonal prolactin specific antibody form a first complex.

Second incubation: After addition of a monoclonal prolactin specific antibody labeled with a ruthenium complex and streptavidin-coated microparticles, a sandwich complex is formed and becomes bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. The analyzer automatically calculates the analyte concentration of each sample (either in μIU/mL, ng/mL or in mIU/L).

Helicobacter pylori Ags
The H. pylori Antigen Rapid Test Cassette (Feces) is a rapid chromatographic immunoassay for the detection of H. pylori antigens in human feces specimens. In this test, the membrane is pre-coated with anti-H. pylori antibodies on the test line region of the test. During testing, the specimen reacts with the particle coated with anti-H. pylori antibodies.

RESULTS
This Case-control laboratory based study was done in Khartoum State from women attending El Ingaz Medical Center.

The age range of the infected females was 15-45 years, 28 infected females were between 15-25 years representing 56%, followed by 19 in the age interval 26-35 years representing 38% and the last were 3 infected females between 36-45 years representing 6% only. There was no statistical significant difference in serum prolactin levels between infected females at different age groups (P.value 0.761). Result is illustrated in (Table-1).

The effect of a recurrent infection with H. pylori on serum prolactin levels among study population (Figure-1). 7 infected females (14%) had a recurrent infection with H. pylori, 43 infected females (86%) had no recurrent infection with H. pylori, and (P.value 0.078) as shows in (Table-2) .There was no statistical significant difference in serum prolactin levels between infected females with and without a recurrent infection with H. pylori.

Regarding to serum prolactin in cases according to the duration of the disease duration range was 1 to 3 months with (P.value 0.574) shows in (Table-3).

The distribution of the serum prolactin concentrations relative to the reference range (Figure-2). 24 (48%) of infected females was found to have a serum prolactin concentration within the reference range and 26 (52%) of infected females have a serum prolactin concentration were exceeded the target range.

Prolactin concentration was higher in the patients compared with controls. In this study hyperprolactinemia was reported in 52% of the patients. As shows in (Table-4) statistical analysis showed that there was significant difference in the mean of prolactin concentration between infected and uninfected females group (P.value 0.000). Regarding correlation between prolactin concentration and subject’s age, H. pylori infection duration or recurrence of infection, no significant was found to be observed.

Table-1: Mean of serum prolactin in cases at difference age groups

<table>
<thead>
<tr>
<th>Age groups</th>
<th>N</th>
<th>%</th>
<th>P.value</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 - 25 Year</td>
<td>28</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>26 - 35 Year</td>
<td>19</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>36 - 45 Year</td>
<td>3</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100</td>
<td>0.761</td>
</tr>
</tbody>
</table>
Table-2: Mean of serum prolactin in cases according to recurrent infection with H.pylori:

<table>
<thead>
<tr>
<th>History</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>7</td>
<td>559.00</td>
<td>79.356</td>
<td>0.078</td>
</tr>
<tr>
<td>No</td>
<td>43</td>
<td>481.33</td>
<td>108.992</td>
<td></td>
</tr>
</tbody>
</table>

Table-3: Mean of serum prolactin in cases according to the duration of disease:

<table>
<thead>
<tr>
<th>Duration</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than month</td>
<td>31</td>
<td>479.61</td>
<td>112.618</td>
<td>0.574</td>
</tr>
<tr>
<td>From 1 - 2 month</td>
<td>13</td>
<td>516.00</td>
<td>104.432</td>
<td></td>
</tr>
<tr>
<td>From 2 - 3 month</td>
<td>6</td>
<td>505.67</td>
<td>98.299</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>492.20</td>
<td>108.141</td>
<td></td>
</tr>
</tbody>
</table>

Table-4: Mean of serum prolactin in cases and controls:

<table>
<thead>
<tr>
<th>Study groups</th>
<th>Number</th>
<th>Mean</th>
<th>SD</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prolactin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases</td>
<td>50</td>
<td>492.20</td>
<td>108.141</td>
<td>0.000</td>
</tr>
<tr>
<td>Controls</td>
<td>50</td>
<td>312.08</td>
<td>104.700</td>
<td></td>
</tr>
</tbody>
</table>

Fig-1: The effect of a recurrent infection with *H.pylori* on serum prolactin levels among study population

Fig-2: Distribution of Prolactin levels among infected females

**DISCUSSIONS**

This Case-control laboratory based study was carried out on 50 infected females with *Helicobacter pylori* infection (cases) and 50 apparently healthy individual with negative *H. pylori* test (control) at different ages and at same sex (women) to determine the effect of *H. pylori* infection on serum prolactin level. The age range of the infected females was 15-45 years. 28 infected females (56%) were between 15-25 years, 19 infected females (38%) were between 26-35 years and only 3 cases (6%) were between 36-45 years. The age range of controls was similar to age range of the study group, 5 (10%) of them had taken medication for *Helicobacter pylori* and 45 (90%) of them haven’t taken medication for *H. pylori* and the duration of disease was taken in this study at duration range 1 to 3.
month, 7 of infected females (14%) had recurrent infection with \textit{H. pylori}.

The results reveal that prolactin concentrations were significantly higher in the infected females compared with controls; this indicates that \textit{H. pylori} infection has an effect on the serum prolactin levels with \((P \text{ value}<0.0001)\), there was no study so far in literature search analyzing variable.

Steroid hormones including steroid pre-hormone, pregnenolone (PN), dehydro -epiandrosterone (DEA), and epiandrosterone (EA) possess a hydroxyl group (3 beta- OH) with beta-configuration at the carbon-3 position of steroid framework, as with free cholesterol (FC). Our study agree with who examined the capability of \textit{H. pylori} to assimilate the 3 beta -OH steroid hormones, and conclude the relationship between Steroid Hormones and \textit{Helicobacter pylori} [18].

There is no statistical significant between \textit{H. pylori} infection and prolactin concentration according to age , that may be due to the narrowing age group was selected in this study ( fertility age from 15 to 45 years) also may due to un balanced selection of numbers of cases and controls at any age groups. Also there was no statistical significant difference in serum prolactin levels between infected females among duration of disease, this may attribute to that, this study at the narrow duration of disease, that to confirm all women included in this study hadn’t any reproductive problems before this study.

There was no any statistical significant difference in serum prolactin levels between infected females with and without a recurrent infection with \textit{H. pylori}. There was - to our knowledge- no study so far analyzing this relationship.

CONCLUSION
This study concluded that prolactin concentration was significantly higher in the infected females compared with controls. However, hence no similar studies were found; more studies in the study population are needed to support the findings. A recurrent infection with \textit{H. pylori}, age, and disease duration are not appeared any significant differences in serum prolactin levels between infected and uninfected females.

RECOMMENDATIONS
More studies including a larger sample size and equally distributed variables, women take contraceptives drugs or had any reproductive problem must be excluded to confirm these results. Also more studies including other types of endocrine assay tests could be done.

Females with \textit{H. pylori} infections could attend fertility centers or any hospitals to perform prolactin test and other reproductive hormones measurements.

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REFERENCES


