Sudan University for Science and Technology

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Frequency of *Helicobacter.pylori ure*A gene From Fecal Specimens using (Polymerase chain reaction)

معدل تردد جين يوريا A لجرثومه الملويه البوابيه من عينات البراز بواسطه تفاعل سلسله البلوميرات

A Dissertation Submitted in the Partial Fulfillment for the Requirements of M.Sc. in Medical Laboratory Science (Microbiology)

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الايه

(وَمَا تَوْفِيقِي إِلاَّ بِاللهِ عَلَيْهِ تَوَكَّلْتُ وَإِلَيْهِ أُنِيبُ)

صدق الله العظيم

(سورة هود الاية 88)

الاهداء

الي من بلغ الرسالة.... وادي الامانة ...ونصح الامة...الي نبي الرحمة ونور العالمين

الي من نذرت عمرها في تربيتنا بلا فتور او كلل .امد الله في عمرها بالصالحات

امي الحبيبة

الي من كلل العرق جبينه ...وشققت الايام يديه ...الي من علمني الصبر والعزيمه .البسك الله ثوب العافية

ابي الحبيب

الي الانسان الذي علقت علية امالي في اجتياز درب الحياة الطويل ...رفيق دربي

زوجي العزيز

الي فلذات كبدي...وقرة عيني ....حفظكما الله من كل مكروة وجعلكما من عباده الصالحين

ابني محمد واحمد

الي من تحليا بالاخاء...وتميزا بالوفاء ...الي من اسعد بهما ومعهما وفقكما الله

أخي محمد ومعتصم

الي الذي كلما أظلم الطريق امامنا لجأنا الية فانار لنا الطريق ..الي من علمنا حب العلم جزاه الله خيرا

خالي الصادق وزوجتة حسنات

الي كل الرائعين من حولي ....الي الذين يحترقون لكي يضيئوا للاخرين نورا.

الي كل هولاء اهدي عصارة جهدي فعذرا اذا لم اجد غير هذا الجهد .

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ABSTRACT

This study aimed to detect *Helicobacter pylori ureA* gene in fecal specimen using PCR among patient attended to Saad Rishwan health center, Omdurman, from May, 2017 to March 2018.

Personal and clinical data were collected by questionnaire after verbal consent, stool samples were collected.immenochrometography test for stool *Helicobacter pylori* antigen was performed, then polymerase chain reaction was used to detect *ureA* gene

It was found that 37(61.7%),23(38.3%) are positive to ICT for female and male respectively .

Out of 60 patients positive for *H.pylori* stool Ag ,7 (11. 7%) of them were positive for PCR .

This study concluded that detection of *H.pylori* using fecal specimens

Without removal of inhibiters may give false negative result

And the use of *ureA* primers for detection of *H.pylori* may be less sensitive than others genes. Also the high sensitivity of *H.pylori* stool antigen test my give false positive.

ملخص الدراسة

هدفت هذه الدراسة للكشف عن معدل تردد جين يوريا A لجرثومه الملويه البوابيه من عينات البراز بواسطه تفاعل سلسله البلوميرات بين المرضي في مركز سعد رشوان الصحي في الفترة ما بين مايو 2017 الي مارس 2018.

تم جمع المعلومات الشخصيه والطبية عن طريق الاستبيان بعد موافقة المرضي الشفوية ومن ثم اخذت عينات البرازوتم فحص جرثومة المعدة وتم استخلاص الحمض النووي وفحصت عن طريق جهاز تفاعل البلمرة السلسلي(PCR).

من مجموع 60 مريضا نتيجتهم ايجابيه باستخدام الاجسام المضادة لبكتريا جرثومه المعدة في البراز(stool Ag) 7(11,7%) منهم كانت نتيجتهم ايجابية لجين يوريا A لجرثومه الملويه البوابيه باستخدام الجين *ure*A

وقد وجدت هذة الدراسة ان معدل حدوث بكتريا جرثومة المعدة في الاناث اكثر من الذكور باستخدام الاجسام المضادة لبكتريا جرثومه المعدة في البراز(stool Ag)

وخلصت هذة الدراسة ان استخدام عينات البراز من غير ازاله المثبطات وفحصها بجهاز تفاعل البلمرة السلسلي(PCR) قد يعطي نتيجة خاطئةايجابيه زائفه .كما ان استخدام الجين *ure*Aقد يكون اقل حساسية من استخدام الجينات الاخري.كما ان الحساسية العالية لفحص جرثومة المعده من البرازباستخدام (stool Ag) قد يعطي نتيجةايجابيه خاطئه.

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Abbreviation

Cag: cytotoxin-associated gene

DNA:Deoxy ribo nucleic acid

ELISA: Enzyme- linked immno sorbent assay

H.pylori: Helicobacter pylori

IgG:Immnoglobulin G

IgM: Immunoglobulin M

LPS: lipolysaccharide

PPI: proton pump inhibiter

Vac: Vacuolating cyto toxin

WHO: world health organization

Chapter One

Introduction

1.1 INTRODUCTION

Helicobacter pylori is a microaerophilic, spiral shaped Gram negative bacterium that colonizes the human stomach. It has been linked to chronic active gastritis, peptic ulcers disease, gastric cancer, and mucosa-associated lymphoid tissue lymphoma (Marshall and Windsor, 2005). *H. pylori* has been classified as a definite class I carcinogen by the World Health Organization (Palli et al., 2007)

The prevalence of *H. pylori* infection is 25%-50%in developed countries and 70%-90% in developing countries (Kabir, 2001) the most probable mode of transmission is person to person spread but oral-oral and fecal – oral transmission have also been reported)

Various tests have been developed to diagnose the infection. *H.pylori* can detected by noninvasive and invasive methods the latter requiring endoscopy. Noninvasive testing for *H.pylori* can be done by measuring exhaled C13 of labelled CO2 (known as urea breath test UBT.) by serology, and by analysing body materials such as faeces, saliva ,and urine .However positive results obtained by serology do not necessarily indicate current infection by *H.pylori* .UBT requires an expensive instrument such as a mass spectrometer ,which not always available in routine clinical laboratories. Because of ease and availability. Faeces can be convenient samples for detection of *H .pylori.* Faecal testing is appropriate for children as faeces can be obtained from them without their active collaboration, as compared with samples collected by endoscopy or for UBT .Few techniques such as bacterial culture, PCR and enzymes immunoassay have been used to detect *H.pylori* in faeces.

1-2: Rational

Helicobacter pylori has been linked to chronic active gastritis, peptic ulcers disease, gastric cancer, and mucosa-associated lymphoid tissue lymphoma. *H. pylori* has been classified as a definite class I carcinogen by the World Health Organization. And prevalence of *H.pylori* infection may exceed 70% in some developing countries (Kabir, 2001).

Invasive and non-invasive techniques are used for diagnosis of *H. pylori* infection. Invasive methods such as histology, rapid urease test (RUT), microbiological culture and polymerase chain reaction (PCR). Non-invasive tests include stool antigen test, serology and urea breath test (UBT). Some factors which influence the choice of a given testing strategy include sensitivity, specificity, the clinical circumstances and the cost-effectiveness of the test (Peng et al ., 2009) Molecular methods such as PCR have the capability to sensitively and accurately determine both the presence of infection and the genotype of bacteria . Because of ease and availability faeces can be convenient samples for detection of *H.pylori* . for all this reasons this research was conducted.

1.3 Objectives

1.3.1 General objective

To detect frequency of *Helicobacter pylori ureA* gene in fecal specimens by PCR.

1.3.2 Specific objectives

1-To detect *H.pylori* in stool specimens using ICT.

2-To detect the presence of *H. pylori urea A gene i*n fecal specimens from symptomatic patients by PCR.

Chapter Two

Literature Review

LITERATURE REVIEW

2.1-History

In 1979 Robin Warren, a pathologist in Perth, Western Australia, began to notice that curved bacteria often were present in gastric biopsy specimens submitted for histological examination. These organisms were not present within the gastric mucosa but were present in the mucus layer overlying the tissue (Marshall et al.,1989). Warren found that similar organisms had been described by European pathologists in the late 19th century, but because they could never be isolated, they were ignored and ultimately forgotten by generations of physicians and scientists. A young trainee in internal medicine, Barry Marshall, became interested in Warren's observations, and together the two sought to isolate the organisms from biopsy specimens. Since the organisms had the appearance of curved, gram-negative rods, the investigators used methods for the isolation of *Campylobacter* species, which involved inoculating the biopsy specimens onto selective media and incubating the cultures under micro aerobic conditions. Since most campylobacters grow within 48 h under such conditions, plates without visible grow within 48 h under such conditions, plates without visible growth were discarded within 3 days. The initial cultures from approximately 30 patients were negative, but by chance one culture was incubated for 5 days over an Easter holiday and colonies were seen (Marshall,1989) Subsequently become clear that *H. pylori* infection was strongly associated with the presence of inflammation in the gastric mucosa (chronic superficial gastritis) and especially with polymorph nuclear cell infiltration (chronic active gastritis). Subsequently, organisms were isolated from 11 patients; the organism was characterized and called *Campylobacter pyloridis* (now known as *Helic obacter pylori).*

2.2 Microbiological characteristic

H. pylori has morphologic and growth similarities to the campylobacters, with which they were originally classified. The cells are slender, curved rods with polar flagella. The cell wall structure is typical of other Gram-negative bacteria, although Helicobacter LPS may be less toxic than its enteric counterparts. Growth requires a microaerophilic atmosphere and is slow (3 to 5 days). A number of unique bacteriologic features have been found in *H. pylori.* The most distinctive is a urease whose action allows the organism to persist in low pH environments by the generation of ammonia. The urease is produced in amounts so great (6% of bacterial protein) that its action can be demonstrated within minutes of placing *H. pylori* in the presence of urea. Another secreted protein called the(vacuolating cytotoxin VacA). causes apoptosis in eukaryotic cells it enters generating multiple large cytoplasmic vacuoles. The vacuoles are felt to be generated by the toxin's formation of channels in lysosomal and endosomal membranes .

Most *H. pylori* strains also contain a 30 gene PAI, so called because the guanine cytosine content of the PAI differs from the rest of the genome. This suggests the PAI is a genetic cassette acquired from some unknown organism in the distant past. Most of the PAI genes code for elements of a contact secretion system, which in other bacteria transfers DNA or proteins across the outer membrane to the extracellular space or into other cells. The cells receiving the products of these secretion systems include bacterial, plant, and epithelial cells. In *H. pylori*, the secretion system injects *VacA* and a protein *Cag*, also coded in the PAI, into epithelial cells. Once in the cell, *Cag* induces changes in multiple cellular proteins and has a strong association with virulence (Ryan and Ray,2004).

2-3 Epidemiology

Although *H. pylori* infection is common, the precise mechanism underlying its transmission is not definitively established. Suggested methods of transmission include ingestion of contaminated water (Aziz et al., 2015), fecal-oral means (Bui et al., 2016). and even periodontal pockets serving as a reservoir (Lauritano et al.,2015) . Other possibilities include transmission by flies (Cave, 1997), iatrogenic spread with unsterile endoscopes and use of pH probes . Most children in developing countries are infected. Before age 10 years, but in the US and other developed countries infection more commonly occurs in adulthood. *H. pylori* infection is less common in developed countries with a prevalence of approximately20-30% .In developing countries disease burden is higher with prevalence rates approaching 90% (Dunn and cohem, 1997).

2-4 Diagnosis

2-4-1 Invasive Methods

2-4-1-1 Rapid Urease Test

The Rapid Urease Test (RUT) is a popular invasive diagnostic *H. pylori* test that is relatively quick, cheap and simple to perform. It detects the presence of urease in or on the gastric mucosa. Best results for RUT are obtained if biopsies are taken from both the antrum and corpus. The biopsy used for RUT can also be used for other tests such as for molecular-based tests of microbial susceptibility or for host factors (Uotani and Grraham, 2015) false positive results are rarely observed. However, treatment with proton pump inhibitors or antimicrobial agents, which prevent the growth of urease producing *H. pylori* prior to the RUT, may. Cause false negative results. Moreover, the sensitivity of the test in patients with peptic ulcer disease with bleeding as

well as in patients with intestinal metaplasia is significantly lower (Lee and Kim,2015). Therefore in these cases a negative RUT result should be complemented by a second method.

2.4.1.2 Histology

The presence of typical spiral motile bacteria accompanied by inflammatory reaction in the histopathological sections of stomach was the first described method used for diagnosis of the *H. pylori* (Lee and kim, 2015).

Along with routinely applied staining like Giemsa, hematoxylin, and eosin, there are some more specific staining procedures which facilitate the diagnosis of *H. pylori* infection. However, the accuracy of the histo-pathological diagnosis of *H. pylori* always depends on the number and the location of collected biopsy materials. While *H. pylori* can be detected in even a single biopsy taken from the correct site, to achieve a higher sensitivity, multiple biopsies are recommended. Moreover, the possible presence of other bacterial species with a similar morphology to *H. pylori* in the stomach (Patel *et al* ., 2005) can be another source of error which negatively affects the accuracy of the test. In addition, treatment with proton pump inhibitors (PPI) or antibiotics prior to sampling may transform the shape of *H. pylori* to a coccoid form. Although molecular biology techniques like *in situ* hybridization (Patel *et al*., 2014) or immunological methods (Wang *et al*., 2014) are helpful solutions for the pitfalls mentioned above, the long assay time of between three hours and three days and the requirement of a relatively complex infrastructure for these methods compromise routine application.

2-4-1-3 Culture

Although it should be stated that *H. pylori* culture is not a routine procedure in initial diagnosis, in many bacteriology laboratories *H. pylori* isolation via the culture of biopsy samples is a routine second line approach (Samuels et al., 2000). remains challenging. This technique, although highly specific, is not as sensitive as other tests like histology and the rapid urease test. As well as for purposes of scientific research, cultured live *H. pylori* is used for diagnostic approaches and for the detection of antibiotic resistance if treatment failure is suspected .(Taj *et a*l ., 2003).

H. pylori requires a microaerophilic atmosphere (5% to 10% oxygen, 5% to 12% carbon dioxide and 80%–90% nitrogen with humidity) and a complex culture media. The most commonly used media contains Brucella, Columbia Wilkins-Chalgren, brain-heart infusion or trypticase agar bases, supplemented with sheep or horse blood (Ndip *et al*., 2003). Because isolation of this microaerophilic organism from. gastric biopsy specimens takes a long time, up to 5–7 days, to overcome the problem of growth of other competitors that exist in the sample, the culture media is supplemented with specific antibiotics. Although *H. pylori* could be cultured from stool specimens (Namavar *et al.*, 1995) due to the presence and growth of numerous other bacteria and especially microorganisms phenotypically similar to *H. pylori*, colonies have to be further characterized by other methods. Moreover, it is possible that the bacterium goes into a viable form that cannot be cultured (coccoid form) which leads to false negative results (Azevedo *et al*., 2007) .

2-4-1-4 PCR

PCR based detection of *H. pylori* could be categorized under invasive as well as non-invasive methods.

Molecular diagnostics have dramatically changed the clinical management of many infectious diseases in the past decades. PCR currently remains the best developed molecular technique as it provides a wide range of clinical applications, including specific or broad-spectrum pathogen detection, evaluation of emerging novel infections, surveillance, early detection of bio threat agent, and antimicrobial resistance profiling (Yang et al., 2004). While PCR could be applied for the detection of *H. pylori* in biopsies, this technique is more qualified for its use in samples taken from the oral cavity or from stool. PCR-based techniques applied as a non-invasive approach (*i.e.*, from stool samples) tend to be more cost effective than other, if traditional methods. In addition to the improved specifications of this technology like high sensitivity and specificity, simplicity, and automated procedures, there are several other advantages, to be considered. Practically, regardless of genome size, any genomic material could be used as a template sample for PCR, which allows sampling from multiple origins (Rimbara et al ., 2013). The high efficiency of this method also achieves fast results. Since antibiotic resistance is currently the major challenge in microbiology, it has to be pointed out that the fast acquirement of results of not only the diagnosis of *H. pylori* but also of its susceptibility to the right antibiotics is extremely important. There are a couple of PCR methods like multiplex PCR which have been employed and recommended as an alternative to culture for resistance testing to antibiotics like clarithromycin .However, a limitation lays in the fact that PCR cannot detect metronidazole resistance. Here, bacterial culture would still be necessary to cover this pitfall especially if a resistance to antibiotics is suspected or again in the case of second line therapies.

The achievement of the criteria mentioned above with its advantages is possible through different PCR approaches. In addition to the conventional PCR methods, a new approach like the colorimetric detection of *H. pylori* DNA using isothermal helicase-dependent amplification is a valuable tool. The rapid application of the test is complemented by reasonably high (up to 95%) sensitivity and specificity of the assay (Gill et al., 2008).

Despite the numerous advantages of PCR-based techniques, there are still some challenges regarding its appropriate application in diagnostics. While correct sampling and preparation of samples prior to assay is a common issue in diagnostic assay, sensitivity and specificity of PCR-based assay are strongly dependent on the design of the method. For instance, due to the extraordinary variability of *H. pylori* genome, the selection of the target gene and PCR primer pairs dramatically influence specificity and sensitivity of the test (Lu et al.,1999). As the whole genome of most known pathogens including many *H.* pylori strains has been successfully sequenced in the last decade, it is very important to design and select the PCR primers based on a comprehensive bioinformatics analysis of relevant genomes. Here, it is mandatory to design primers according to genomic sequences that are highly conserved in all *H. pylori s*train.

The relevance of several specific *H. pylori* genes has been studied in the past. *vacA* encodes a vacuolating cytotoxin which is excreted by *H pylori* and damages epithelial cells.(Leunk ,*1997)*The gene is present in all strains, and comprises two variable parts.

The s region (encoding the signal peptide) is located at the 59 end of the gene and exists as a s1 or s2 allele. Within type s1 several subtypes (s1a, s1b, and s1c) can be distinguished. The m-region (middle) occurs as a m1 or m2 allele. The mosaic combination of s- and mregion allelic types

determines the production of the cytotoxin and is associated with pathogenicity of the bacterium. (Atherton et al.,1997) .

2-4-2 Non-Invasive Methods

2-4-2-1 Urease Breath Test

The urease breath test (UBT) is one of the most common non-invasive tests used. This non-invasive test, available in different versions, has been evaluated in different studies, showing high sensitivity,specificity and accuracy (Braden et al., 1994). The test is able to detect the infection indirectly by measuring the existence of bacterial urease produced by *H. pylori* in the stomach. There are different types of this test comprising 13C- or 14C-isotope labelled urea. If *H. pylori* is present, the urease hydrolyses the labelled urea and the exhaled isotope containing ammonia can be detected applying the samples to a measuring device. This test is recommended by the Maastricht IV/Florence Consensus Report as a valuable diagnostic tool for the detection of infection and for therapy control (Malfertheiner et al., 2012). It has been shown that UBT can distinguish an ongoing from a past infection; hence, it is able to detect the eradication progress after treatment (Gatta et al.,2003). A recent study was conducted comparing C13-UBT and a monoclonal *H. pylori* stool antigen test in infants and toddlers in South America (Gatta et al .,2003). The study suggests that both tests are reliable for *H. pylori* diagnosis in very young patients because of the high concordance between test results. In comparison, a study performed in Turkey evaluating C14-UBT in elderly patients also showed good diagnostic parameters with a sensitivity and specificity of 91.4% and 93.8%, respectively, compared to histopathology In recent years, different application protocols and detection devices were developed. A recently published study describes a

residual gas analyzer-based mass spectrometry approach with possible point of-care application (Maity et al., 2014). A further study has evaluated a low dose capsule-based UBT approach compared to conventional UBT and invasive tests . This approach seems to be superior to the conventional UBT in terms of sensitivity and specificity, resulting in 100% sensitivity and specificity but depending on the time of sample collection One of the limitations is the presence of other urease producing bacteria in the stomach, e.g.

H. heilmannii, which might lead to false positive results. Moreover, acute bleeding and co-medication can lead to false negative test results (Bravo et al.,1999). For reasons of required fasting prior to taking the test, it might be onerous for the patient. In addition, because of the detection devices needed, costs tend to be higher compared to alternative tests used (Masucci,2013). Furthermore, according to different protocols available, the accuracy of UBT test results depends on the amount of urea applied, the point in time in which samples are taken and the set point of he cut off value.

2-4-2-2 Fecal Antigen Test

Fecal antigen tests detect antigens in stool samples. ELISA formats comprising monoclonal antibodies against *H. pylori* proteins showed improved results compared to polyclonal approaches (Paimela et al., 2006) .The current guideline evaluates the use of the stool antigen test as equivalent to the UBT if a validated laboratory-based monoclonal antibody is used(Malfertheiner et al.,2012) compared the diagnostic accuracy of five different stool antigen tests in adult dyspeptic patients comparing monoclonal enzyme immunoassay tests (EIA) and rapid immune chromatographic assays (ICA). The sensitivity and specificity of the tests

analysed had a high variation between 48.9%–92.2% and 88.9%–94.4%, respectively, depending on the test format. They conclude that EIA tests are more accurate compared to the currently available ICA based test, that are fast and easy to use but provide less reliable results (Korkmaz et al., 2013). A recent meta-analysis conducted by Zhou and colleagues analysed forty-five studies, including 5931 patients and evaluated the test performance of a *H. pylori* stool antigen test in children. The average sensitivity and specificity was 92.1% and 94.1%, respectively (Zhou et al.,2013). Furthermore, the available stool antigen tests have been shown to be able to distinguish infected from treated patients (Gisbert et al.,2002), enabling the confirmation of treatment.Degradation of antigens in the intestine and consequent disintegration of epitopes might lead to false negative results. Moreover, the process of sample handling could be fastidious for patients. False negative results may occur when the bacterial load is low, due to proton-pump inhibitors or the recent use of antibiotics or bismuth (Manes et al;2001).

2-4-2-3 *Serological* Test

Immune responses against *H. pylori* are utilized to detect infection by analysing patients' blood or serum for IgG and IgA antibodies. Serology is the only test which is not affected by those local changes in the stomach that could lead to a low bacterial load and to false negative results (Malfertheiner et al;2012). According to guidelines proposed by the Maastricht conference, only IgG detection is considered and the favored method is ELISA. Currently, different formats of serological tests are available, including simple ELISAs that use whole lysates or recombinant produced *H. pylori* proteins as antigens. . More recently, immno-blots (Veijola et al.,2008) luminex-based bead assays (Gao et al.,2009)) and line assays (Formichella et al.,2013) were developed; these allow amore

specific evaluation of the infecting *H. pylori* strain in terms of bacterial virulence factors and host immune responses towards the human pathogen. Moreover, they show improved sensitivity and specificity (Formichella et al.,2013),(Rahman et al .,2009) because additional and highly purified antigens are included. These non-invasive tests are easy and cheap to perform. The potential for developing a rapid diagnostic test makes serology an interesting option for testing populations in areas with little or no access to medical facilities. Using an automated approach, large cohorts could be tested within a short time, allowing population based studies. Good diagnostic parameters in terms of robustness, sensitivity and specificity are given as shown in different studies (Formichella et al., 2013). These studies show that improved serological test formats lead to increased sensitivity of over 95% and specificity ranging from 85%–96%, making serology a preferable alternative to other non-invasive tests. However, there are also contradictory studies with low sensitivity and specificity values for the serological test (Mahmood et al.,2010) probably due to the test format applied or the antigens used.

Risk stratification might be an interesting issue, detecting combined antibody responses to different virulence factors. Promising studies have analysed different *H. pylori* antigens, showing significant correlations between positive immune responses and clinical outcomes like chronic atrophic gastritis, intestinal metaplasia, dysplasia, and gastric cancer (Song et al.,2014). In particular, there have been great improvements by applying single defined recombinant *H. pylori* antigens; the well-characterized oncogenic protein *CagA* that has been shown to correlate with severe gastric disorders and *VacA* that are in clinical use (Adamsson et al., 2013).

The immune responses towards VacA could be even more specific by differentiating between m1 and m2 variants of VacA (Ghose et al .,2007) .

More recently, antigens like NapA, GroEL, HyuA have been shown to have the potential to be used as biomarkers to predict infected individuals having a higher risk of developing premalignant changes that are a hallmark of gastric cancer

development (Liu et al., 2014). The combination and evaluation of different biomarkers adapted to the region where they are used might be the future method for ruling out patients at higher risk of developing severe diseases.

Antibody responses towards antigens are sustained for a long period after eradication therapy, as shown in different studies (Ho and marshall,2000). These studies argue that a confirmation of treatment success by serology is not applicable for this reason. However, while some immune responses to certain antigens persist for a long period of time (especially CagA), for some antigens the antibody titre decreases within a short period, also depending on the Ig-class tested (Kato et al; 1999). This phenomenon could also be utilized as clinical readout to confirm treatment success by analysing the decline of antibody responses, as shown in different studies (Yunoki et al., 2000). conclude from their study that it would be the reasonable and even perhaps preferred method of monitoring H. pylori infections (Wang et al., 2014)

2-4-2-4Rapid Diagnostic Test

Rapid Diagnostic Test Despite numerous publications on rapid diagnostic testing (RDT) methodologies for infectious diseases, such testing has become neither commonplace nor an integral component of serviceaaaaQ 11s offered by clinical microbiology laboratories (Lennox et al., 2015). However, because of the emerging need for rapid diagnosis and treatment of virulent strains of different viral and bacterial infections, discussions

regarding routine application of RDT are increasing within current medical circles. Debate on the value of RDT has broadened and continues to increasingly encompass new infection Results of RDT testing become available within a couple of minutes to a few hours. A clinical specimen is processed in a few steps (preferably in a single step) at the site where it is collected (point of care). The quality and value of the RDT is determined by its sensitivity and specificity, the time required for results, and its cost and availability. According to the guidelines set forth by the Maastricht conference, it appears that those antigens with either high or low molecular weight are more specific (Malfertheiner et al., 2012).

There are currently many H. pylori RDT kits commercially available. However, how far these tests fit in with standard clinical practice is still undetermined.

As generally in microbiology laboratories, H. pylori RDT kits could also be performed based on the following four approaches: (A) testing of H. pylori specific antigens; (B) molecular detection of the specific H. pylori nucleic acid sequence; (C) rapid biochemical reaction test and (D) serologic detection of H. pylori specific antibodies. Regardless of which approach is applied, these tests have to be validated based on gold standards. In addition to specificity and sensitivity, the value of the overall agreement with the gold standard (biopsy or culture) plays a crucial role in the evaluation of the kit. Although the majority of the approved RDTs for H. pylori reach a non-ideal sensitivity and specificity (in general < 90%), there are promising findings that lead to significant improvement of the assays. For instance, in the process of testing of H. pylori specific antigens, H. pylori specific antibodies are required. While antibodies produced by immunization with whole H. pylori lysate or a mixture of bacterial compartments are more prone to be sensitive, they reduce the specificity of

the test. On the other hand, antibodies which are produced against single molecules are very specific, although they gain more false negative results. The same argument is also valid in other sero-immunology based approaches that aim at detecting H. pylori specific antibodies. While utilization of single conserved molecule of H. pylori leads to reasonable specificity, this assay may lead to higher false negative results compared to the application of a mixture of antigens, which in turn may cause higher false positive results. As mentioned above, new promising studies have described new H. pylori antigens which have been successfully tested in preliminary assays. For example, FliD, a hook flagellar protein of H. pylori, is a highly conserved molecule in all H. pylori species. While this molecule has no or only low homology to other bacterial species which prevents cross reactivity, it has shown a strong antigenicity in animal studies. Assays which employ this protein as antigen in the diagnosis of anti H. pylori antibodies have shown high sensitivity and specificity (Khalifeh et al., 2013) Serologic tests should therefore include antigens for sensitivity, specificity testing, and antigens that identify infections by more pathogenic H. pylori strains, which might provide the basis for decisions about further treatment. (Malfertheiner et al.,2012).

5-2 -Treatment

Multiple options exist for the treatment of patients with active H. pylori infection . Determining which regimen is most appropriate depends on several factors including local susceptibility patterns, whether a patient is undergoing initial treatment, likelihood of patient adherence, and patient factors such as the presence or absence of drug allergies. Adherence to therapy is an important factor with non-adherence contributing to treatment failure as well as antibiotic resistance. As a result, regimens that are “easier” to take are generally preferred.

Eradication regimens that require the patient to take drugs twice a day are more likely to be followed when compared to more frequent dosing regimens. Both three and four drug regimens are acceptable frst-line therapies to eradicate H. pylori . Triple therapy consists of a PPI, clarithromycin, and amoxicillin or metronidazole and is taken twice daily. Triple therapy should be administered for 14 days (Yuan et al., 2013). Quadruple therapy utilizes a PPI or H2 receptor antagonist, bismuth, metronidazole, and tetracycline. These medications are taken up to four times daily for 10–14 days (Chey and wong ,2007). Although both triple and quadruple therapies have been recommended as first-line options for the treatment of H. Pylori, the complexity of four times daily dosing and the high pill count in quadruple therapy may be challenging for patients to take and has the potential to be associated with decreased patient adherence.

6-2 Prevention of H.pylori

In general practice good hygiene by frequently washing hand and properly preparing food and complete full course of treatment if diagnosed with H.pylori all this can reduce the infection by H.pylori

Effort to find an effective method for non-antibiotic control of H.pylori infection are therefore urgently required .Some strain of lactobacillus and Biffidobactrium can inhibit H.pylori growth .A vaccine can be used either prophylactically or therapeutically.

Chapter Three

Material and Methods

MATERIAL AND METHODS.

3-1 Study design

This was a cross-sectional descriptive study.

3-2 Study area

The study was conducted at Saad Rishwan Heath Center ,Omdurman, Khartoum State

3-3 Study period

The study was carried out during the period from May, 2017 to March 2018.

3-4 Study population

Sixty patients with *H.pylori* positive ICT stool antigen.patiants were males and females of different ages.

3-5 Sampling

Non probability sampling -Quota population was selected randomly

3-6 Sample size

Sixty fecal specimens.

3-7 Ethical consideration

Permission to carry out the study was obtained from the college of graduate studies, Sudan university of Science and Technology.

All patients were informed for the purpose of the study before collection of the sample and verbal consent was taken from them.

3-8 Data collection

A questionnaire including personal and clinical data was used

3-9 Sample processing

Sixty stool samples from positive stool *H.pylori* antigen were collected in containers contain normal saline .Then stored at -20 until used.

3-10-Laboratory work

3.10.1 Fecal processing

For ICT(1-2ml or1-2g) of feces was collected then the specimens collection tube holed up right and opened the cap onto the specimens collection tube then inverted and 2fulldropof the extracted specimens was transferred to specimens wells of test cassette then the results was read at 10 minutes.

Principle of immnochrmotogrophy test:

This is a qualitative flow immunoassay test for detection of *H.*pylori antigens in human feces specimens .the membrane is pre coated with anti H.pylori antibodies on test line region .the specimens reacted with particle coated with antibodies .the mixture migrates upward on membrane by capillary action to reacted with anti H.pylori antibodies on the membrane and generate a red line .

Interpretation of results

Positive: two lines appeared one red line for control line region and the another red line for appeared in test region

Negative: one line appeared in control line region

Invalid: control line failed to appeared.

3-10-2 DNA extraction

One gram of stool was dissolved in 1ml of Guanidine Hydrochloride (57,2gram dissolved in100ml D.W),300ul of NH4acetate (57.81gram dissolved in 100ml DW) and 10ul of proteinase K was added then the samples were incubated over night at 37c. After overnight incubation the samples cooled at room temperature ,and then 2ml of pre-chilled chloroform was added, after that samples were centrifuged for 5 min at 6000 rpm .

After overnight incubation samples were centrifuged for 10 min at 6000 rpm then the supernatant was drained .Upper layer was collected to a new falcon tube, 10ml of cold Absolute Ethanol was added to collected samples, then kept at -2oc over night

Pellet was washed with 4ml of 70% ethanol, and then was centrifuged for 10 at 6000rpm.

Supernatant was poured off and pellet was allowed to dry

Then dissolved in 100 ul of ddH2O, and then was incubated at 4c

Preparation of 10x tris Base EDTA (TBE) buffer:

Amont of108 g of tris basewas added to 55 g of boric acid and 40 ml of 0.5%of EDTA and then dissolved into 1L of DW.

Prepration of 10x TBE buffer:

10ml of 10x were added to 90 ml D.W and mixed

Preparation of ethidium bromide:

10 mg of ethidium bromide were dissolved into 0.5ml D.W and kept into brown bottle toaviod light .

Preparation of agarose gel :

Amount of 0.75g of agarose powder were dissolved into100 ml of 10x TBE,then the mixed had been cooled .then 2ul of ethidium bromide was added ,mixed well and poured in a casting tray that had been tapped up appropriately and equipped with spacers and suitable comb to from wells ,any bubbles were removed and the gel allowed setting at room temperature after solidification the comb and the spacers wee gently removed.

3-10-3-PCR amplification .

Samples were amplified using PCR machine (HealForce,K960,China). PCR for detection ureaA gene of *H.pylori* was performed using maxi me PCR pre mix kit (iNRO Biotechnology, Korea) and primer (Macrogen, Korea)

ureA -197F (5-AACCGGATGATGTGATGGAT3-) and urea-413R (5-GGTCTGTCGCCAACATTTTT3-)

Amplification was performed in 20ul reaction volumes containing 17ul of ddH2O, 1ul of primer and 2ul of DNA template .PCR condition for urea gene was performed as follow initial denaturation at 94C for 3 min ,followed by 40 cycle of denaturation at 94C for 3 sec ,annealing at 58C for 30 sec ,and extension at 72Cfor 40 sec ,with final extension at 72C for 5min .then PCR products were subjected to electrophoresis on a2%agarose gel.

Visualization of PCR product:

The amplicons were separated at 120 V for 30 min in a 1.5%(wt/vol) agarose gel containing ethidium bromide ,bands were visualized under U.V trans illuminator (U vitec –UK) to detect the specific ampilied products by comparing with 50 base pairs standard ladder (INtRon Biothochnology, Seongnam, Korea).

3-11-Data analysis

The statistical analysis of the result was performed using the Statistical Package for Social Science (SPSS) for windows version 16 .

Chapter Four

Results

RESULTS

4-1 Frequency of *H.pylori* infection by stool Ag:

H.pylori stool Ag	Frequency	Percent
Positive	60	100%
Negative	0	0%
Total	60	100%

Detection of *H.pylori* from fecal specimens by PCR

Out of 60 patients 7 (11.7%) of them were positive for *H.pylori* by PCR while 53(88,3) of them were negative table 4-1

Table 4-2 Frequency of *H.pylori ureA* gene by PCR

PCR	Frequency	Percent
Positive	7	11.7%
Negative	53	88,3%
Total	60	100%

Table 4-3 Frequency of *H.pylori* infection detected by stool test Ag according to gender

Gender	Frequency	Percent
Male	23	38.3%
female	37	61.7%
Total	60	100%

Chapter Five

Discussion, Conclusion and Recommendations

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

The detection of *H. pylori i*nfection will greatly contribute to further improvement of the health management of *H. pylori* associated disorders. This study aimed to use PCR for detection of *H.pylori* from fecal specimens. From sixty positive stool antigen for *H.pylori* seven of them showed positive result for *H.pylori ureA* gene the low sensitivity may due to type of stool specimens which contain PCR inhibiter thus may give false negative result (widjoatmodjo et al.,1992)

The finding in the present study are in agreement with those of vanzwet(1994) which used PCR for detection *H.pylori* in fecal specimens without removal of inhibiter which give false negative results .

The finding in the present study are in agreement with those of vanzwet(1994) which used PCR for detection *H.pylori* in fecal specimens without removal of inhibiter which give false negative results .

In contrast to this study result.the sensitivity of PCR for fecal specimens after removal of inhibiters is 95%, according to studies performed in India (KABIR,2001). and 73% according to willam(1999)

Also the high sensitivity of *H.pylori* stool antigen test my gave false positive result in another study HPSA displayed high sensitivity (89.5%) for diagnosis of *H.pylori* infection but low specificity (77.8%) (forne etal., 2000).

The use of *ureA* primers for detection of *H.pylori* was less sensitive than 16SrRNA and *cagA* primers in detecting *H.pylori* possibly because of lower quantities of ureA RNA with in each bacterial cell, these finding were consistent with hypothesis that the amount of *H.pylori* urease

production in vivo may be low (Blaser,(1993).this may be the causes of low sensitivity of this study result.

Out of 60 samples ,37(61,7%) were females and 23(38,3)were males Frequency of females were higher than males this agree with study done in Sudan Imtithal et al (2017) also agree with study done by Seyda et al (2007). inTurkey.

5.2 Conclusion

Detection of *H.pylori* ureA gene using fecal specimens without removal of inhibiters may give false negative result. And The use of *ure*A primers for detection of H.pylori may be less sensitive than others genes

5.2 Recommendations

1-Removal the PCR inhibitors from fecal specimens when using Guanidine hydrochloride protocol to extract DNA

2-Large sample size is critical for better result.

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Appendixes

APPENDIXES

University of Sudan for Sciences& Technology

Faculty of Medical laboratory Sciences

Frequency of *Helicobacter pylori ureA* gene in fecal specimens

Using PCR

No ( )

1. Name

……

…….

.2-Gender: male( ) female ( ).

1.Lab Results:

ICT for *helicobacter pylori* Positive ( ) Negative( )

*H.*pylori ureA gene Positive ( ) Negative( )