

Non-Invasive Vs Invasive Procedures For Detection of H. Pylori Infection in Patients With Gastric Symptoms in Alkharj, Saudi Arabia

Authors

Ibrahim M. Abdel Aziz^{1,2}, Shafquat Qamar³, Mohamed M. Shaaban⁴, Sanaa Kamal¹

¹Medicine Department, Salman Bin Abdul Aziz College of Medicine, Al Kharj, Saudi Arabia

²Department of Gastroenterology & Infectious Diseases Al Azhar Faculty of Medicine, Cairo, Egypt

³Microbiology Department, Salman Bin Abdul Aziz College of Medicine, Al Kharj, Saudi Arabia

⁴Anatomy Department, Salman Bin Abdul Aziz College of Medicine, Al Kharj, Saudi Arabia

Corresponding Author

Ibrahim M. Abdel Aziz

Professor of Gastroenterology & Hepatology

Salman Bin Abdul Aziz University Hospital, College of Medicine, Al Kharj, Saudi Arabia

Email-dribra1011@gmail.com, 00966537243359

ABSTRACT

Objectives: *H. pylori* is detectable in nearly 100% of adult patients with duodenal ulcer and about 80% of patients with gastric ulcer. An association between *H. pylori* and gastric cancer is confirmed. In developing countries, where most children become infected by the age of 10, gastric cancer rates are very high. Diagnosis of *Helicobacter pylori* is achieved by invasive or non-invasive methods such as upper endoscopy, antral biopsies, *H. pylori* PCR of antral biopsies, *H. pylori* ELISA and *H. pylori* antigen detection in stool through ELISA technique. The aim of the current study is to compare the diagnostic performance of invasive and non-invasive diagnostic methods in the Kingdom of Saudi Arabia.

Design & Methods: Patients between with dyspeptic symptoms were enrolled from the Salman Bin Abdel Aziz University Hospital, Al Kharj, Saudi Arabia between Oct/2013 and Sept/2014. Patients responded to a questionnaire to investigate possible dyspeptic symptoms and then underwent. Besides the gastric biopsy, established as the gold standard test, the stool ELISA test and *H. pylori* ELISA serology were also applied.

Results: The diagnostic performance of the HP stool antigen assay was as follows (Table 2): sensitivity of

94.5%, specificity, 96.2%; positive-predictive value, 93%; negative-predictive value, 94%; and concordance of 90.4%. The diagnostic performance of the *H. pylori* ELISA assay was also high, with a sensitivity of 90.5% and specificity of 92%. Combining the HP stool antigen with the ELISA assay raised the sensitivity to 98%, the specificity to 97%, PPV to 96% and NPV to 95%.

Conclusions: The ROC curve showed a good correlation between the compared methods. The standardization of the ELISA test for the detection of *H. pylori* in stool specimens constitutes a non-invasive diagnostic alternative.

Key Words: *Helicobacter pylori*, serology, stool antigen test, endoscopy

INTRODUCTION

Helicobacter pylori infection is a worldwide problem. It is the most common cause of chronic gastritis, and is strongly linked to peptic ulcer disease and gastric cancer [1]. A strong association has been reported between *H. pylori* infection and gastric lymphoma and adenocarcinoma of the body and antrum of the stomach particularly lymphoid tissue lymphomas (MALTomas) [2,3]. Furthermore, *H. pylori* infection is seemingly involved in the pathogenesis of several extragastric diseases, such as mucosa-associated gastroesophageal reflux disease (GERD) [4,5], coronaritis [6], iron deficiency anemia [7]. To date, such associations are still uncertain, and causality related to these associations needs to be proved.

H. pylori is one of the most common bacterial infectious agents with prevalence rates ranging between (29% and 90% of cases) [8,9]. *H. pylori* infection occurs more frequently in developing countries than in industrialized countries. *H. pylori* strains differ in their potential to cause diseases [8,9]. Moreover, the acquisition of

H. pylori seems to occur at higher rates in developing countries with prevalence rates that differ from one country to another and may differ between different ethnic, social, or age groups within the same country [9].

H. pylori infection can be diagnosed by either invasive or non-invasive methods. Invasive techniques based endoscopy includes, CLO tests, culture, histology, direct gram stain and PCR-based methods. Non-invasive methods of detection include serology, *Helicobacter pylori* stool antigen (HpSA) test and urea breath test (UBT) [10,11]. Immunoglobulin A (IgA) and IgG serologic tests have been evaluated in several studies but the reliability and cut-off value have not been confirmed. Some studies suggested the use of IgM as an indicator of active disease [12-14] while others have found IgM to have little diagnostic utility [15,16]. The HpSA test has been reported to be as reliable as the UBT for diagnosis as well as for monitoring *H. pylori* eradication albeit at a lower sensitivity [17,18]. Although culture is considered the gold standard it is not often used for the detection of *H. pylori*, it is sometime not possible to perform either due

to clinical problems preventing endoscopy, or patients' refusal, or insufficient endoscopic or laboratory facilities. Among different geographical regions, the prevalence of *H. pylori* in the Kingdom of Saudi Arabia is between 70-90%⁽¹⁹⁾. Since the prevalence of *H. pylori* is still high, feasible diagnostic noninvasive tests are required for the determination of diagnosis and follow-up after eradication treatment. Therefore, it is crucial to optimize non-invasive methods for reliable diagnosis of *Helicobacter pylori* infection and for post-therapy assessment. Therefore, the current study is designed to analyze the utility and diagnostic performance of a panel of non-invasive tests in comparison to endoscopy for the diagnosis and monitoring of *H. pylori* eradication among a population with gastric manifestations in Al Kharj, Central Saudi Arabia.

PATIENTS & METHODS

A total of 100 outpatients complaining of gastroduodenal disorders and dyspepsia were enrolled in this study from Oct 2013 and Sept 2014. At presentation no patient had previous specific therapy for *Helicobacter pylori*. Patients with a history of antibiotics, proton pump inhibitors, bismuth within one month prior to endoscopy, serology or HpSA test. Patients with previous gastric surgery, and previous diagnosis of gastric cancer were also excluded from the study.

All patients were subjected to clinical examination and were assessed for eligibility to

gastrointestinal endoscopy. Informed consent was obtained from all patients prior to participation in this study and before any procedure. The Institutional Review Board of the University of Salman Bin Abdel Aziz University approved this study. The study was conducted according to the principles of the 1974 Declaration of Helsinki.

HELICOBACTER PYLORI STOOL ANTIGEN ASSAY:

Stool specimens were collected and stored at -20°C upon arrival at the laboratory after DNA extraction was performed. The stool was thawed for stool antigen assay, and subsequently stored again at -20°C for future retesting. The *Helicobacter pylori* Antigen, EIA, Stool Test (Quest Diagnostics, New Jersey, USA) was used according to the manufacturer's instructions. The assay was based on S-ICT, using a single monoclonal antibody against *H. pylori* flagellin antigens. In brief, a plastic bar was used to add 500 mg to 1 g fecal sample to a vial containing 1 mL buffer. After gentle vortexing, the fecal sample was emulsified. Two to four drops of emulsified stool sample were placed in the sample port of the test cassette. The test was interpreted after 15 min at room temperature. The appearance of a red line in the reading window indicated a positive result, with the positive control band that was also red in color. A positive result (antigen detected) is indicative of *H. pylori* presence. A negative result

(antigen not detected) indicates absence of *H pylori* or an antigenic level below the assay limit of detection. The test has a sensitivity and specificity of 96% for detecting *H pylori* infection. False-negative results may be obtained on specimens from patients who have ingested selected medications (antimicrobials, proton pump inhibitors, bismuth preparations) within the 2 weeks prior to specimen collection. Serology was also performed using an enzyme-linked immunosorbent assay (IgG and IgA using *Helicobacter pylori* IgG, ELISA Kit (MBS580106) (Biosource, Heidelberg Germany according to the manufacturer's instructions. Briefly, Diluted patient serum is added to wells coated with purified antigen. IgG specific antibody, if present, binds to the antigen. All unbound materials are washed away and the enzyme conjugate is added to bind to the antibody-antigen complex, if present. Excess enzyme conjugate is washed off and substrate is added. The plate is incubated to allow the hydrolysis of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgG specific antibody in the sample. Eligible patients were referred to upper endoscopy at King Khalid Hospital, Al Kharj, KSA. Upper endoscopy was performed for 45 patients. Detailed endoscopic examination was performed and several biopsies were taken for histological examination. Biopsy specimens were prepared for histological examination by staining with

hematoxylin-eosin and Warthin-Starry silver stains; diagnoses were made using the Sydney classification⁽¹⁷⁾. At least one biopsy specimen had been obtained from the antrum, with additional specimens obtained from other sites. The grade of *H. pylori* presence was recorded as follows: mild, few focal areas of bacteria; moderate, bacteria in several areas; and severe, abundant bacteria in most glands. The presence of atrophic gastritis, intestinal metaplasia, lymphoid aggregates and/or follicles, gastric ulcers, and ulcer scars was also recorded.

The diagnosis of *H. pylori* infection was established by the concordance of 2 or more positive test results from the 3 tests performed (i.e., histology, HP stool antigen or HP ELISA). *H. pylori* infection status was classified as follows: definite positive, all 3 tests show positive results, or a positive histology result and either of the other tests shows a positive result; probable positive, positive results by stool antigen assay and HP ELISA; and negative, all 3 tests are negative.

STATISTICAL ANALYSIS

Continuous variables were compared using Student's *t*-test or the Mann-Whitney U test when appropriate. The frequencies were compared using the chi-square test or the Fisher's test if the expected frequency for any cell was five or lower. Results are expressed as mean values \pm SD, P-values and 95% confidence intervals or median and IQR as

appropriate. The Z test was used to compare proportions between groups. Statistical analysis was done using Statistical Analysis Software version 16 (SAS Institute, Inc., NC, USA).

RESULTS

Among the 100 enrolled patients, the prevalence of *H. pylori* infection in males and females was 47.9 and 59.4, respectively. The median age of the patients was 42 years (range, 26–51 years). The prevalence of *H. pylori* infection in patients < 40 and > 40 years was 33.3 and 55.2, respectively ($p=0.04$).

The endoscopic results showed the following oesophageal lesions: gastroesophageal reflux disease (GERD) in 26 (26%) patients, reflux esophagitis in 21 (21%) patient, and Barrett's esophagus in 2 (2%) patients. The rates of positive histological diagnoses of *H. pylori* infection by the number of specimens were as follows: 1 specimen, 31.5 (45/143); 2 specimens, 44.9 (22/49); and 3 or more specimens, 29.4 (5/17). The endoscopic results with respect to the gastric mucosa were as follows: normal in 7 (7%); atrophic gastritis in 23 patients (23%), erosive gastritis in 19 (19%) patients; erythematous gastritis in 8 (8%) patients; combination of atrophic and erosive gastritis, 26 (26%) patients; combination of erosive and erythematous gastritis in 7 (7%) patients; combination of atrophic and erythematous gastritis in 14 (14%) patients and ulcer in 17 (17%) of cases. The histological results revealed chronic active gastritis in 48

(48%), atrophic gastritis in 37(37%), lymphoid aggregation and/or follicle in 38 (38%) and intestinal metaplasia in 6 patients (6%). The prevalence of *H. pylori* infection by disease is as follows: reflux esophagitis 15/21; peptic ulcer in 13/17 patients; intestinal metaplasia in 5/6 patiente; atrophic gastritis in 19/23 patients.

Among the 100 patients, 63, 20, and 17 had definite positive, probable positive and absolute negative *H. pylori* infection status. The diagnostic performance of the HP stool antigen assay was as follows (Table 3): sensitivity of 94.5%, specificity, 96.2%; positive-predictive value, 93%; negative-predictive value, 94%; and concordance of 90.4%. The diagnostic performance of the *H. pylori* ELISA assay was also high, with a sensitivity of 90.5% and specificity of 92%. Combining the HP stool antigen with the ELISA assay raised the sensitivity to 98%, the specificity to 97%, PPV to 96% and NPV to 95%.

Table 1: Baseline Demographic And Clinical Characteristics Of Patients:

Parameter	
Age (years) mean±SD	42.17±6.2
(95% CI of the mean)	(35.86-38.48)
Females (n ;%)	37 (37)
Symptoms (heart burn, dyspepsia, epigastric pain; n, %)	78 (78)
Mean total bilirubin ± SD (mg/dl) (95% CI of the mean)	1.04±1.2 0.6 to 3.5)
Mean ALT ± SD (U/liter) (95% CI of the mean)	44.5±13.96 (12.6 to 61.37)
Mean AST ± SD (U/liter) (95% CI of the mean)	43.15±12.2 (9.94 to 64.26)

Table 2: Helicobacter Pylori Detection By Histology, HP Stool Antigen And ELISA Method

Number of patients	Diagnosis	Histology	HP stool antigen	HP ELISA
63	HP definite positive	+	+	+
20	probable	+	+	-
17	Negative	-	-	-

Table 3: Predictive Value of HP stool antigen and HP ELISA versus Histology:

Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
HP stool antigen	0.94 (0.44–0.96)	0.96 (0.86–0.98)	0.93 (0.77–0.98)
HP ELISA	0.90 (0.74–0.96)	0.96 (0.80–1.00)	0.88 (0.53–0.99)
HP stool antigen + ELISA	0.98 (0.45-100)	0.98 (0.50–1.00)	96 (0.45-0.96)
			0.95 (0.67-0.99)

DISCUSSION

The stool antigen test for *H. pylori* diagnosis has been approved and standardized for use in primary diagnosis and monitoring after treatment. In addition, the Maastricht III Consensus report recommends the use of both the urea breath test and stool antigen test for the diagnosis and follow-up of *H. pylori* infection (20, 21). However, the stool antigen test is non-invasive, cost effective, and requires a short time to perform. Therefore, it is convenient for patients and can be easily performed even in small laboratories and primary outpatient clinics. On the other hand, the HP ELISA requires specific detection equipment which delays the diagnostic process in addition it is more expensive (23). The prevalence of *H. pylori* infection in the present study is similar to that in previous reports of *Helicobacter pylori* in the Kingdom of Saudi Arabia (24,25). The sensitivity and specificity of the HP stool antigen are similar that of a systematic view of 89 studies, which determined sensitivities of 91 and 93 (7). Although it is recommended to test multiple stool specimens in batches (21), only one sample was used for each patient in this study, which may have decreased the sensitivity by 5–10. Although the infection rate was high when 2 specimens were obtained, it was not significantly higher than that determined with 1, or 3 or more specimens. A previous study reported that people > 40 years of age showed higher positive rates of *H.*

pylori infection (5). In the current study, there was significant differences between the prevalence of *H. pylori* in patients > and <40 years of age.

The diagnostic performance of the HP stool antigen assay was as follows (Table 2): sensitivity of 94.5%, specificity, 96.2%; positive-predictive value, 93%; negative-predictive value, 94%; and concordance of 90.4%. The diagnostic performance of the *H. pylori* ELISA assay was also high, with a sensitivity of 90.5% and specificity of 92%. Combining the HP stool antigen with the ELISA assay raised the sensitivity to 98%, the specificity to 97%, PPV to 96% and NPV to 95%. Previous studies reported that combining more than one non-invasive assay performed better. The results indicated that the performance of the evaluated kit was robust, regardless of the histological findings. Unlike the rapid urease test, which has been reported to have low sensitivity in atrophic gastritis and intestinal metaplasia (19,25), the present study showed 81%–100% sensitivity and specificity in cases of atrophic gastritis, intestinal metaplasia, chronic active gastritis, and intestinal metaplasia. GERD has been reported to be inversely correlated with *H. pylori* infection rates (25). In the present study, the prevalence of *H. pylori* infection showed an inverse correlation between these 2 groups, but the performance of the evaluated kit produced similar results. The inclusion of patients with ulcers may have

affected the diagnostic performance of the test in the present and previous studies. Therefore, guidelines for establishing the ratio of patients with and without ulcers to be included in evaluations of stool antigen tests may be required to reduce selection bias.

Combinations of at least 2 tests have been adopted (26), including histology, HP stool antigen, and HP ELISA. The tests used in the present study are different from those used in previous studies, including the urea breath test, culture, rapid urease test on biopsy specimens, serological testing, and histology as the gold standard. Our results confirm that HP stool antigen, and HP ELISA. Assays are non-invasive rapid test for *H. pylori* diagnosis that demonstrates high performance among patients with dyspepsia.

IN CONCLUSION

Our study showed a good correlation between the compared methods. The combination of the *H. pylori* stools antigen and *H. pylori* ELISA test for the detection of *H. pylori* in stool specimens constitutes a non-invasive diagnostic alternative.

ACKNOWLEDGMENTS

The authors extend their appreciation to the deanship of scientific research at Salman Bin Abdul Aziz University, Alkharj, KSA, for their continuous support and encourage us for their valuable scientific research. We also appreciate all clinical physician's and medical secretaries in department of medicine, Salman Bin Abdul Aziz

University hospital, Alkharj City, Saudi Arabia for their continuous helpful and typing this manuscript.

CONFLICT OF INTEREST

SPECIFIC AUTHORS CONTRIBUTIONS:

Professor Ibrahim M Abdel Aziz planned and designed the study, conducted patients' recruitment, clinical assessment, follow-up and data collection, data interpretation, and drafting of the manuscript. Professor Abdel Aziz has approved the final draft submitted.

Professor/ Sanaa Kamal conducted all patients' recruitment, clinical assessment, data interpretation and also statistical reviewers. Professor/ Kamal has approved the final draft submitted.

Dr. Shafquat Qamar performed the serial biochemical studies, Elisa, Stool Antigen, and Culture, interpreted data, drafted the manuscript, and provided important intellectual content. Dr. Qamar has approved the final draft submitted.

Professor/ Mohamed Shaaban had conducted the histologic study of endoscopic biopsies, staining, follow-up data collection and interpretation, and drafting of the manuscript. Professor/ Shaaban has approved the final draft submitted.

KEY POINT

1. H.P is highly endemic especially in developing countries as environmental pollution (water & food hygiene) essential for transmission of such disease.
2. Early detection & diagnosis of *H. P* infection it was very important to decline

the most dangerous consequences (MALTomas).

3. New modalities for the diagnosis of H.P open new hope for early detection and treatment as well for eradication of H.P.
4. With early detection and management of H.P, the dyspeptic symptoms & Gastric lymphoma (MALT) will be decline and quality of life will be improved.

REFERENCES

1. Versalovic, J., Carroll, K., Funke, G., et al. (2011): Manual of Clinical Microbiology. ASM Press, Washington, D.C.
2. Suerbaum, S. and Michetti, P. (2002): Helicobacter pylori infection. N. Engl. J. Med., 347, 1175–1186.
3. Hirschl, A. and Makristathis, A. (2005): Non-invasive Helicobacter pylori diagnosis: stool or breath tests? Dig. Liver Dis., 37, 732–734.
4. Blanco, S., Forne, M., Lacoma, A., et al. (2009): Evaluation of a latex agglutination test (PYLOGEN) for the detection of Helicobacter pylori in stool specimens. Diagn. Microbiol. Infect. Dis., 63, 349–353.
5. Choi, J., Kim, C., Kim, D., et al. (2011): Prospective evaluation of a new stool antigen test for the detection of Helicobacter pylori, in comparison with histology, rapid urease test, C-urea breath test, and serology. J. Gastroenterol. Hepatol., 26, 1053–1059.
6. Cirak, M., Akyon, Y. and Megraud, F. (2007): Diagnosis of Helicobacter pylori . Helicobacter, 12, 4–9.
7. McNulty, C., Lehours, P. and Megraud, F. (2011): Diagnosis of Helicobacter pylori infection. Helicobacter, 16 (Suppl.), 10–18.
8. Veijola, L., Myllyluoma, E., Korpela, R., et al. (2005): Stool antigen tests in the diagnosis of Helicobacter pylori infection before and after eradication therapy. World J. Gastroenterol., 14, 7340–7344.
9. Manes, G., Zanetti, M.V., Piccirillo, M.M., et al. (2005): Accuracy of a new monoclonal stool antigen test in post-eradication assessment of Helicobacter pylori infection: comparison with the polyclonal stool antigen test and urea breath test. Dig. Liver Dis., 37, 751–755.
10. Shimoyama, T., Sawaya, M., Ishiguro, A., et al. (2011): Applicability of a rapid stool antigen test, using monoclonal antibody to catalase, for the management of Helicobacter pylori infection. J. Gastroenterol., 46, 487–491.
11. Suzuki, N., Wakasugi, M., Nakaya, S. et al. (2002): Production and application of new monoclonal antibodies specific for a fecal Helicobacter pylori antigen.
12. Dominguez, J., Forne, M., Blanco, S., et al. (2006): Comparison of a monoclonal with a polyclonal antibody-based enzyme immunoassay stool test in diagnosing Helicobacter pylori Infection before and after eradication therapy. Aliment.

- Pharmacol. Ther., 23, 1735–1740.
13. Deguchi, R., Matsushima, M., Suzuki, T., et al. (2009): Comparison of a monoclonal with a polyclonal antibody-based enzyme immunoassay test in diagnosing *Helicobacter pylori* Infection after eradication therapy. *J. Gastroenterol.*, 47, 713–716
 14. Chisholm, S., Watson, C., Teare, E., et al. (2004): Non-invasive diagnosis of *Helicobacter pylori* infection in adult dyspeptic patients by stool antigen detection: does the rapid immunochromatography test provide a reliable alternative to conventional ELISA kit? *J. Med. Microbiol.*, 53, 532–627.
 15. Trevisani, L., Sartori, S., Rossi, M., et al. (2005): Evaluation of a new rapid immunoassay for the detection of *Helicobacter pylori* in faeces: a prospective pilot study. *Aliment. Pharmacol. Ther.*, 1, 485–489.
 16. Goh, K., Chan, W., Seiji, S., et al. (2011): Epidemiology of *Helicobacter pylori* infection and public health implications. *Helicobacter*, 16 (Suppl.), 1–9.
 17. Dixon, M., Genta, R. and Yardley, J. (1996): Classification and grading of gastritis. The updated Sydney system. International Workshop on the Histopathology of Gastritis. *Am. J. Surg. Pathol.*, 20, 1161–1168.
 18. Gurtner, C., Hirschl, A., Dragosics, B., et al. (2004): Novel real-time PCR assay for detection of *Helicobacter pylori* infection and simultaneous clarithromycin susceptibility testing of stool and biopsy specimens. *J. Clin. Microbiol.*, 42, 4512–4518.
 19. Kim, S., Lee, H., Lee, W., et al. (2011): New allele-specific real-time PCR system for warfarin dose genotyping equipped with an automatic interpretative function that allows rapid, accurate, and user friendly reporting in clinical laboratories. *Thromb. Res.*
 20. Shin, C., Kim, N. and Lee, H. (2009): Validation of diagnostic tests for *Helicobacter pylori* with regard to grade of atrophic gastritis and/or intestinal metaplasia. *Helicobacter*, 14, 512–519.
 21. Malfertheiner, P., Megraud, F., O' Morain, C., et al. (2007): Current concepts in the management of *Helicobacter pylori* infection: the Maastricht III Consensus report. *Gut*, 56, 772–781: 22. 6464
 22. Do, M., Lee, Y., Choi, C., et al. (2009): The changes in prevalence and the related factors of *Helicobacter pylori* infection in Korean health checkup subjects during 8 years. *Korean J. Gastroenterol.*, 53, 76–83.
 23. Gong, Y., Sun, L, Jin, S., et al (2010): Comparative study of serology and histology based detection of *Helicobacter pylori* infections: a large population-based study of 7,241 subjects from China. *Eur. J. Clin. Microbiol. Infect. Dis.*, 29, 907–911.

24. Al Faleh FZ, Ali S, Aljebreen AM, Alhammad E, Abdo AA. Seroprevalence rates of Helicobacter pylori and viral hepatitis A among adolescents in three regions of the Kingdom of Saudi Arabia: is there any correlation? Helicobacter. 2010 Dec;15(6):532-7
25. Jaber SM. The pattern of CagA and VacA proteins in Helicobacter pylori seropositive asymptomatic children in western Saudi Arabia. Saudi Med J. 2005 Sep;26(9):1372-7.
26. Working Party of the European Helicobacter pylori Study Group (1997): Guidelines for clinical trials in Helicobacter pylori infection. Working Party of the European Helicobacter pylori Study Group. Gut, 41, S1–9.

List of Abbreviations:

ELISA: Elisa immunosorbent assay

H. P: Helicobacter pylori

PCR: polymerase chain reaction

PPV: Positive predictive volume

NPV: Negative predictive volume

GERD: Gastroesophageal reflux disease

HpSA: Helicobacter pylori stool antigen

UBT: Urea breathe test.

IgA: Immunoglobulin A.

IgG: Immunoglobulin G.

IgM: Immunoglobulin

KSA: Kingdom of Saudi Arabia