

80ml of distilled water and final volume was made up to 100ml. To it 0.002 gm of phenol red was added, pH was adjusted up to 6.4 to 6.8 by using dilute hydrochloric acid. The broth was sterilized by using 0.22 μ m Millipore filter, and dispensed in aliquot (0.5-1 ml) into a capped polypropylene tubes. The biopsy specimen for the URUT was removed from the biopsy forceps with a sterile toothpick and placed immediately into the polypropylene tube. Particular care was taken not to shake the tube after placing the biopsy into it so that a rapid positive result could be achieved⁽⁴¹⁾. A positive test result was indicated when there was a color change in the medium surrounding the biopsy from yellow to magenta. The test tube was left at room temperature and examined at intervals over 24 h. Convenient times chosen were 1, 5, 10, 20, 30 min and 1, 2, 3 and 24 h after insertion of the biopsy specimen into the urease test reagent.

Presence of H.pylori in the impression smears:

Impression smear was performed from the positive and negative specimen in the URUT test; crushed between two sterilized glass slides; heat fixed; stained with 40% carbolfuchsin for 1 min and examined under an oil immersion lens for the presence of a helical or more strikingly curved bacteria (Figure 1)

Blood samples:

The basal blood samples for assays of IgG antibodies for *H.pylori* and serum Ferritin were drawn after an overnight fast. Class antibodies to *H.pylori* were determined using specific ELISA tests (*Helicobacter Pylori* IgG ELISA Test Kit Cat. No. 601 040.01, Biohit Plc, Helsinki, Finland) according to the Instructions of the manufacturer. Samples with an ELISA value of <34 EIU (EIU=enzyme Immune Units) were

considered negative, and samples with an ELISA value >42 EIU were considered positive. Samples with values between 34-42 EIU (Cut –off ~38 EIU) were considered as Borderline

Serum Ferritin was determined using VIDAS Ferritin (Enzyme Linked Fluorescent Assay Kit Cat.No.30 411, bioMerieux sa) according to the instructions of the manufacturer. Serum Ferritin values were considered as:

Iron deficiency = If concentrations are lower than 20 ng/ml in women and 30 ng/ml in men.

Inflammation = If concentrations are greater than 250 ng/ml in women and 350 ng/ml in men.

Histological evaluation of gastric biopsies

Two antrum biopsies were fixed in formalin and paraffin-embedded, and stained with hematoxylin and eosin; and subsequently evaluated by an experienced pathologist. The degree of inflammation present in the histological specimens was classified according to the updated Sydney system⁽⁴²⁾ (data not shown in this paper). A grading from absent, mild, moderate and severe was assigned for four histological variables: chronic inflammation (mononuclear cell infiltration), activity (polymorphonuclear neutrophil infiltration), glandular atrophy, and intestinal metaplasia.

Definition of H. pylori Infection

The gold standard for classifying a patient as being infected with *H. pylori* (in present study) was either detection the organism in the gastric biopsy by having the Ultra Rapid Urease test /or anti-*H. pylori* antibodies and histology results with or without visualized by microscopic examination. Patients were considered uninfected with *H. pylori* when all tests were negative.