INTRODUCTION
Helicobacter pylori

1-History

Helicobacter pylori were first discovered in the stomachs of patients with gastritis and stomach ulcers in 1982 by Dr. Barry Marshall and Dr. Robin Warren of Perth, Western Australia. At the time, the conventional thinking was that no bacterium can live in the human stomach, as the stomach produced extensive amounts of acid of strength similar to the acid found in a car battery. Marshall and Warren rewrote the textbooks with reference to what causes gastritis and gastric ulcers. In recognition of their discovery, they were awarded the 2005 Nobel Prize in Physiology or Medicine.(1)

Interest in understanding the role of bacteria in stomach diseases was rekindled in the 1970s, with the visualization of bacteria in the stomach of gastric ulcer patients.(2) The bacterium had also been observed in 1979, by Australian pathologist Robin Warren, who did further research on it with Australian physician Barry Marshall beginning in 1981.

After numerous unsuccessful attempts at culturing the bacteria from the stomach, they finally succeeded in visualizing colonies in 1982, when they accidentally left their Petri dishes incubating for 5 days over the Easter weekend., Warren and Marshall find out that most stomach ulcers and gastritis were caused by infection by this bacterium and not by stress or spicy food, as had been assumed before.(3)

The bacterium was initially named *Campylobacter pylori*, then renamed *C. pylori* (pylori being the genitive of pylorus) to correct a Latin grammar error. When 16S ribosomal RNA gene sequencing and other research showed in 1989 that the bacterium did not belong in the genus *Campylobacte*r, it was replaced into the genus, *Helicobacter*. The genus derived from the ancient Greek "spiral" or "coil".(4)

Features that distinguish *H. pylori* from *Campylobacters* are its multiple sheathed flagella, its strong hydrolysis of urea and its unique fatty acid profile (5)

2-Microbiology of *H. pylori* :

Members of the genus *Helicobacter* are all microaerophilic organisms *H. pylori* produce urease, catalase, oxidase, DNase, alkaline phosphotase, leucine aminopeptidase and δ-glutamyl-aminopeptidene and are cephalothin sensitive and resistant (6)

Table (I): Characteristics of helicobacter species.

Helicobacter taxonomy	Source	1ry site	Catalase prod	Nitrate reduc	Alk phosph	Urease	Indoxyl actate hydrolysis	δ-glutamyl transfer	At 42° C	With 1% glycine	Nalidixic acid	Cephalethin	Flagella
H.Bizzozercnii	Human Dog	Stomach	+ve	+ve	+ve	+ve	+ve	+ve	+ve	–ve	R	S	Bipolar
H. Canis	Human Dog	Intestine	–ve	–ve	+ve	–ve	ND	+ve	+ve	ND	S	S	Bipolar
H. Cinaedi	Human Hamster	Intestine	+ve	+ve	–ve	–ve	–ve	–ve	–ve	+ve	S	I	Bipolar
H. Fermallial	Human	Intestine	+ve	–ve	+ve	–ve	–ve	–ve	–ve	–ve	S	I	Bipolar
H. Pullirum	Human Chicken	Intestine	+ve	+ve	–ve	–ve	ND	ND	+ve	–ve	R	S	Mono polar
H. Westmeadii	Human	Unknown	+ve	+ve	–ve	–ve	ND	ND	–ve	–ve	S	R	Mono polar
H. pylori	Human Ca + macaque	Stomach	+ve	–ve	–ve	+ve	+ve	+ve	–ve	+ve	R	S	Mono polar

ND-> not determined. [7]

3-Morphology:

H. pylori are curved or S shaped Gram negative rods 0.5-0.9 µm wide and about 3 µm long. In agar cultures spiral forms are less obvious and organisms appear more as singly curved rods [9]. It's capable of forming biofilms , Electron microscopy shows that the organisms is spiral with blunty ends and 4-8 sheathed unipolar flagella. The flagellar sheath is continuous with the outer membrane of the cell wall and exhibits the typical structure of a membrane [8]. Some flagella have a terminal bulb[9] making it possible for *H. pylori* to more through the thick mucous coat of the stomach lining [10].

A glycocalyx like material surrounding the cell is also apparent [11] *H. pylori* undergoes coccal transformation on exposure to air within 1-2hrs at room tempor after prolonged culture. Coccoid forms are spherical and unflagellate, they appear as U-shaped bacilli with the ends of the two arms joined by a membrane structure. Cocoid forms fail to grow on subculture although they retain the cellular structures that are compatible with the viability and do not appear to be virulent [12]. It may be one of the stages of a putative *H. pylori* biological cycles [13].

H. pylori posses five outer membrane proteins (OMP) families. The largest family includes known and putative adhesions. The other four families include porins, iron transporters, flagellum associated proteins and proteins of unknown function.

Outer membrane proteins (OMPs): a variety of putative outer membrane proteins (OMPs) have been identified in *H. pylori* cell wall, (outer membrane of *H. pylori* consists of phospholipids , lipopalysachrides and cholesterolglucosides). The molecular masses of these OMPs range from 31 to 80 KDa [14].

Urease and HSPB, a homolog of the GroEL protein of Escherichia coli, are abundant in OMP preparations [11].

Several species specific outer membrane proteins have been described including a 19 KDa protein and 26 KDa protein and the flagellar sheath protein 29 KDa. [9].

A family of four protein molecules, designated HOPA, HOPB, HOPC, and HOPD, with apparent molecular masses of 48 to 6 KDa, has been purified and characterized. Each of the proteins forms pores with low single-channel conductance in

a planar lipid bilayer model membrane system. An additional pain molecule, HOPE, has homology to the P2 porin of *Haemophilus influenza* [15].

Some of the OMPS of *H. pylori* are repressible by iron; such proteins may be involved in the uptake of heme from the host. At least one of the iron-repressible proteins (apparent molecular mass, 77 KDa) is immunogenic in infected individuals [11].

The lipopolysachride (LPS): LPS of *H. pylori* has low biological activity, a property which may aid in persistence of infection. Strains of *H. pylori* have a lodder like side chains. These side chains are strains [9].

The O-specific chain of *H. pylori* LPS is fucosylated and mimics Lewis blood group antigens in structure of found on gastric epithelium. Molecular mimicry between *H. pylori* LPS and the host, based on Lewis antigen, may contribute to pathogenesis[16].

Fatty acid profile of *H. pylori* is distinctive and different from the general pattern of *Campylobacters*. *H. pylori* does not possess the methyl substituted methanoquincnes present in campylobacter species [17].

***H. pylori* genomics**

Worldwide variations in the *H. pylori* genome are strongly associated with migration patterns of human populations, suggesting that the first humans were already infected by *H. pylori* as they moved away from East Africa about 60,000 years ago.[18] The first *H. pylori* genome sequence was published in 1997;[19] there are now at least 7 full genome sequences available in the public domain.[20-25] In general, the *H. pylori* genome comprises about 1.6 mega bases, encoding approximately 1,500 predicted open reading frames. About 20–30% of the genome is variable between different strains. This is a relatively high percentage among bacterial species and is thought to result from a high spontaneous mutation rate and a relatively high recombination frequency.[26]

Several highly variable regions within the *H. pylori* genome have been identified including the so-called "plasticity zone" and "cytotoxin associated gene" (*cag*) pathogenicity island. The *cag* island encodes several structural proteins important in assembling a type four secretion system capable of translocating *H. pylori* products (including the immunodominant 120–145 kDa CagA protein) directly inside host gastric epithelial cells.[27] The *H. pylori* genome also encodes several adhesins that are important for ensuring tight contact between *H. pylori* and gastric epithelial cells. These include blood group antigen binding adhesin (BabA) and the sialic acid binding adhesin, SabA. *H. pylori*'s *vacA* gene encodes a multimeric vacuolating exotoxin (VacA), an 88 kDa secretory protein which has the potential of forming intracellular vacuoles in gastric and other epithelial cells.[28] Presence of *vacA* is conserved among all *H. pylori* strains, but the gene exhibits a high level of genetic diversity within regions encoding the signal sequence, intermediate element, and the middle portion of the VacA protein.[29]

4-Growth requirements:

H. pylori is a microaerophilic organism. Its microaerobic requirement will be satisfied by growing in 5-10% O2 and enhanced by CO2 (5-20%)[9].

After laboratory passage some strains become sufficiently aerotolerant that can grow at 10% CO2. *H. pylori* grows poorly if all under anaerobic conditions. Growth occurs at 30° C to 37° C but not at 25° C, variable growth occurs at 42° C [(5)].

H. pylori is nutritionally fastidious and requires complex basal medium with same supplementation so brucella agar, brain heart infusion agar containing either 5 or 10% sheep blood or 7% horse blood, egg yolk emulsion, and cyclodextrin have been used successfully [(30)].

Selective media supplemented with antibiotics that suppress flora or other potential contaminants are available commercially [(31)], and should be used together with non-selective media in order to ensure optimal recovery[(6)].

Growth of *H. pylori* is inhibited by bisulphate in the Campylobacter aerotolerance supplement which consists of famous sulphate, sodium metabisulphite and sodium pyrovate [(32)].

The two main requirements for successful isolation of *H. pylori* are the use of freshly poured plates used without drying and maintenance of adequate humidity throughout incubation [(9)]. *H. pylori* can grow over a wide range of pH 5.5 – 8.5 with good growth between pH 6.9 – 8 [(5)].

Colonies from primary culture take 3-5 days to appear (not discarded before 12 days) and are circular convex and translucent. They do not exceed 2mm in diameter. They are weakly hemolytic on horse blood agar[(9)].

Fig. (1): *H. pylori* growth on blood agar

5-Epidemiology

Prevalence and Incidence

The prevalence of *H. pylori* shows large geographical variations. In various developing countries, more than 80% of the population is *H. pylori* positive, even at young ages [33]. The prevalence of *H. pylori* in industrialized countries generally remains under 40% and is considerably lower in children and adolescents than in adults and elderly people [34].

Within geographical areas, the prevalence of *H. pylori* inversely correlates with socioeconomic status, in relation to living conditions during childhood [34].

In Western countries, the prevalence of this bacterium is often considerably higher among first- and second-generation immigrants from the developing world [35].

While the prevalence of *H. pylori* infection in developing countries remains relatively constant. It is rapidly declining in the industrialized world [36]. The latter is thought to be caused by the reduced chances of childhood infection due to improved hygiene and sanitation and the active elimination of carrier ship via antimicrobial treatment.

Overall, new infection more commonly occurs in childhood and lasts for life unless specifically treated. At least half the world's population are infected by the bacterium, making it the most widespread infection in the world.[37], People infected with it are likely to develop more intense inflammation that may be followed by atrophic gastritis with a higher subsequent risk of gastric ulcer, gastric cancer or both.

A new study clearly illustrates that infection with *H. pylori* occurs in Egypt very early during childhood below the age of 10. It also shows that the detection of *H. pylori* antibodies, in Egypt, is of epidemiological and not clinical utility. [37]

Habitat

The surface of the human stomach mucosa is the major habitat of *H. pylori* , but the organism could be detected in faeces, saliva and dental plaques [38]. *H. pylori* is commonly isolated from non-human primates and stomachs of cats as well [6].

There is a suggestion that water could be a source of the organism but weather the organism merely exist or have some ecological niche in natural waters is unknown [9]. Recent studies suggested that the organism did not posses enzymatic pathways to survive other environments (Winds Prevalence of *H. pylori* varies greatly between geographic locations depending an environmental and socio economic factors.

In developing countries 70-90% of the populations have *H. pylori* infections, white, in developed countries the prevalence is lower ranging from 25-50% [39].

It has been found that the prevalence in Eastern Europe is as high as that in developing countries. In Africa and China 85% of population have evidence of *H. pylori* infection [40].

6-Transmission and Sources of Infection

H. pylori is most likely transmitted from person to person although infection from a common source cannot be rutted out. Support for the person to person transmission carries from evidence of clustering of *H. pylori* infection within families and from reports of custodial institutions and nursing homes [41].Person-Person transmission is possible by three routes:

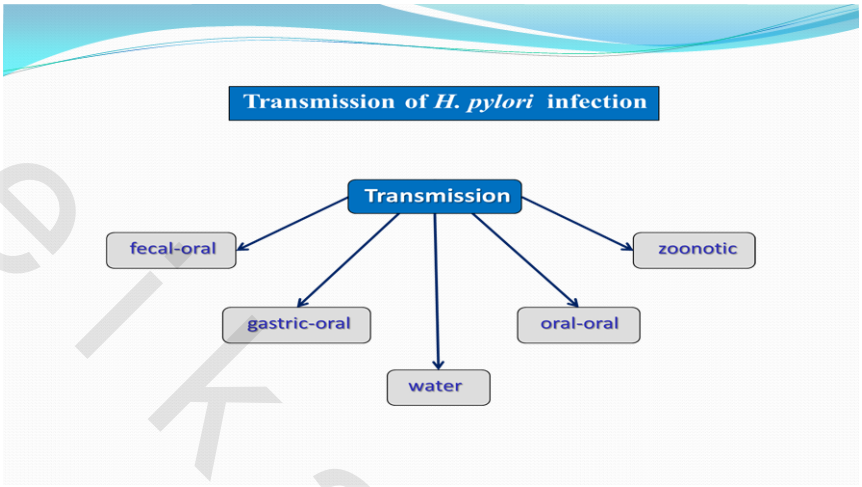

Fig. (2): The modes of transmission of *H. pylori*

Fecal-Oral

Is the most common route. It's indicated that the *H. pylori* shedding in stool is intermittent and feacally contaminated water can also be a source of infection [11].

Oral-Oral transmission

The increased rates of infection among children of West African mothers who premasticate their infants food and among Chinese who share eating utensils favors Oral-Oral transmission [6].

Song et al. found that as small as (1.75 × 10 CFU/ml) of viable *H. pylori* can be cultured from gastric juice. Episodes of gastroesophageal reflux permit access of *H. pylori* to the mouth and the oral cavity has been suggested to be a permanent reservoir for *H. pylori* and can harbor multiple *H. pylori* strains at the same time [42].

Iatrogenic transmission

In which tubes, endoscopes or specimens in contact with the gastric mucosa from one person are introduced to another and this was found to be less common [43].

Sexual transmission

H. pylori infection could be transmitted sexually with the vagina acting as potential temporary or permanent reservoir. The nest recent reservoir suggested for *H. pylori* is the housefly [44]. Although *H. pylori* has been isolated from domestic cats. Several studies have suggested sheep as a possible source of *H. pylori* transmission, a hypothesis that deserves additional investigation [44].

Risk factors

Age

In under developed countries, there is no age related difference in seropositivity, where around 80% of individual <5 years old being infected. By contrast in developed countries few infections occur during childhood and increase in prevalence with age at a rate of about 0.5-1% year [(45)].

Sex

Most studies suggest that males and females are infected at approximately the same rates although in at least one study male sex was significant risk factor for infection [(46)].

Socioeconomic status

The difference in prevalence between developed and developing countries seems to be linked to socioeconomic factors. Poor hygiene, crowded conditions and low level of education are strictly associated with higher infection rates [(44)].

Race and ethnicity

Prevalence also varies between populations of different ethnic origin. In USA healthy adults Hispanic and black populations have seropositivity rates several folds higher than non-hispanic white population independently of other demographic characteristics [(47)].

The cause of this difference is suggested to be due to large interested susceptibility to infection in addition to environmental related factor. Moreover, recently investigators have shown different *H. pylori* genes having distinct racial distribution. [(48)]. Some studies demonstrated that vacA S1c *H. pylori* strains are found exclusively in persons of Asian descent and the study of Gold et al, 2001[(49)] found 90% of Hispanics had similar *H. pylori* strains (vacA, S1b, ml) and all Asian Canadian children were infected by strains with vacA SIC genotype.

In Europe, a distribution gradient of the vacA S1 subtypes have been observed. vacA S1a genotype was more common in individuals from Northern Europe (England, Ireland and Scotland), whereas in Central and South America virtually all *H. pylori* strains contained vacA S1b genotype and in East Asia subtype S1c is more frequently found [(49)].

Occupation

Occupation may influence the prevalence of *H. pylori* infection. Gastroenterologists and endoscopy personnel are at higher risk of *H. pylori* infection.[(50)]

Genetics

Individual susceptibility to *H. pylori* may have a significant impact in acquisitation or clearance of infection. It was found that HLA-DQA1, DIO2 was higher among negative subjects whereas HLA-DQA1, O3Ol was higher in *H. pylori* positive individuals [(51)], while (kulcsarova et al, 2001) [(52)] suggested that HLA-CW6 plays a role in the susceptibly to *H. pylori* infection.

Also some studies have strain that people with blood group A or O are have likely to be infected with the organism as such individuals possess specific receptors for *H. pylori* adhesion on their gastric epithelial cells [46].

7-Aspects of *H. pylori*-associated diseases:

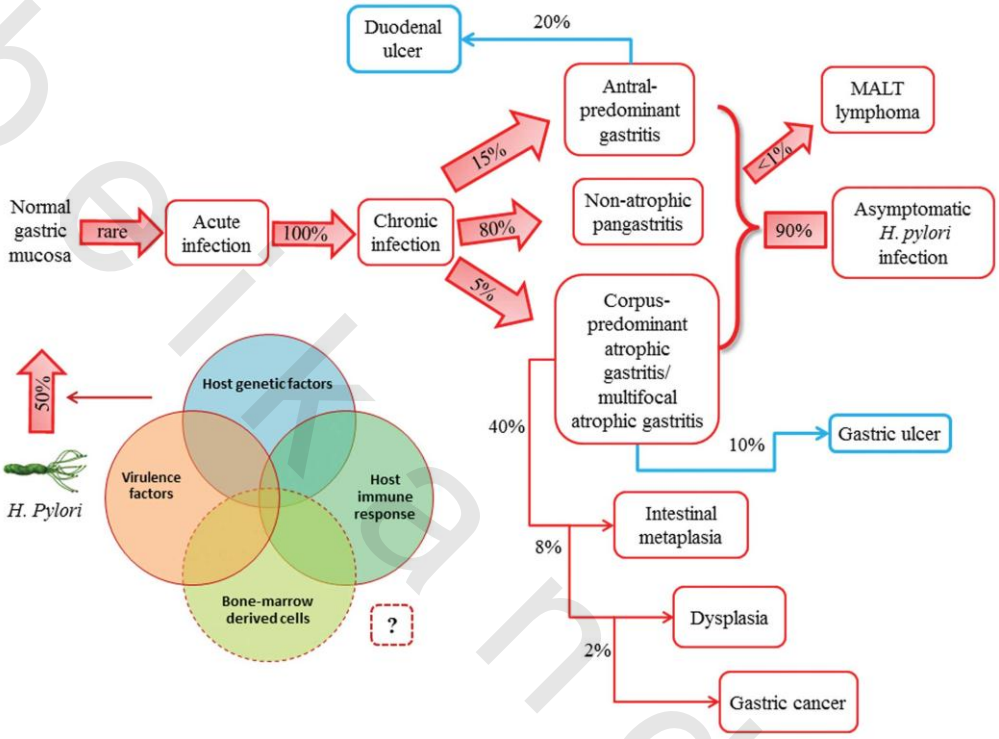

Fig. (3): Illustrating the clinical outcome of H. pylori

Colonization with *H. pylori* is not a disease in itself, but it affects the relative risk of various clinical disorders of the upper gastrointestinal tract and possibly the hepatobiliary tract.

H. pylori positive patients have a 10 to 20% lifetime risk of developing ulcer disease and a 1 to 2% risk of developing distal gastric cancer.[53]The risk of development of these disorders in the presence of *H. pylori* infection depends on a variety of bacterial, host, and environmental factors that mostly relate to the pattern and severity of gastritis.

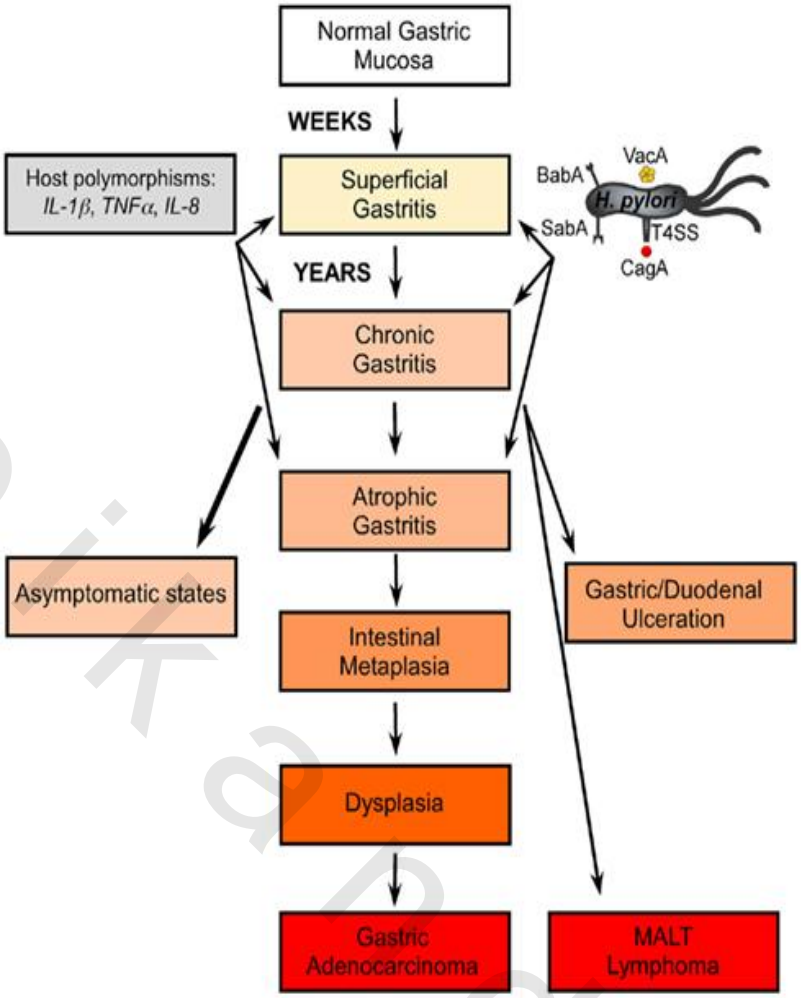

Fig. (4): Schematic representation of aspects of clinical diseases caused by *H. pylori* infection.

Acute gastritis:

The acute phase of colonization with *H. pylori* may be associated with transient dyspeptic symptoms, such as fullness, nausea, and vomiting, and with considerable inflammation of both the proximal and distal stomach mucosa, or pangastritis. This phase is often associated with hypochlorhydria, which can last for months.

Chronic gastritis:

When colonization does become persistent, close correlation exists between the level of acid secretion and the distribution of gastritis. Subjects in whom acid secretion is impaired, due to whatever mechanism, have a more even distribution of bacteria in antrum and corpus, and bacteria in the corpus are in closer contact with the mucosa, leading to a corpus-predominant pangastritis[54].

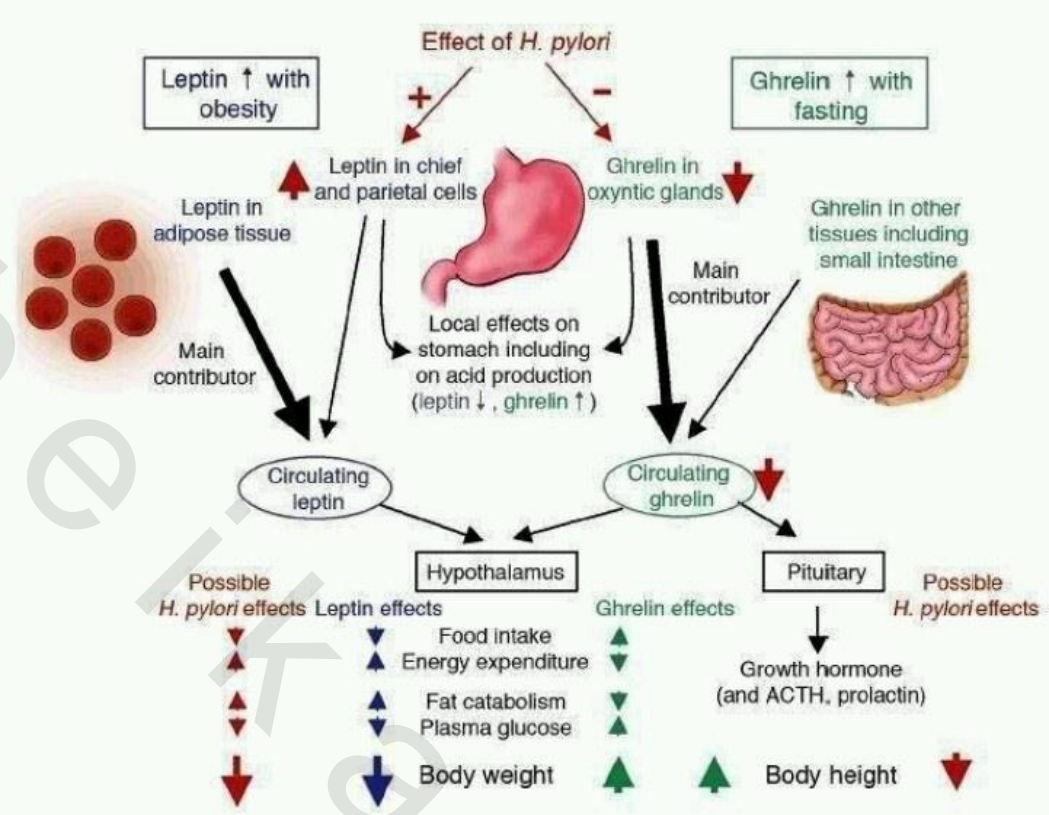

Fig. (5): Schematic representation of the effect of *H. pylori* on the patients organs and disease outcome in *H. pylori* infection.

Peptic ulcer Disease:

Gastric or duodenal ulcers (commonly referred to as peptic ulcers) are defined as mucosal defects with a diameter of at least 0.5 cm penetrating through the muscularis mucosa .Gastric ulcers mostly occur along the lesser curvature of the stomach, in particular, at the transition from corpus to antrum mucosa [(55)]. Duodenal ulcers usually occur in the duodenal bulb, which is the area most exposed to gastric acid.

In Western countries, duodenal ulcers are approximately four fold more common than gastric ulcers; elsewhere, gastric ulcers are more common.

Ulcer complications:

Complications of ulcer disease include bleeding, perforation, and stricture formation. Bleeding is the most common complication of ulcer disease and is estimated to occur in 15 to 20% of ulcers. Approximately 40% of patients presenting with upper gastrointestinal bleeding have a bleeding ulcer.

Atrophic gastritis, intestinal metaplasia, and gastric cancer

Chronic *H. pylori*-induced inflammation can eventually lead to loss of the normal gastric mucosal architecture, with destruction of gastric glands and replacement by fibrosis and intestinal- type epithelium. This process of atrophic gastritis and intestinal metaplasia occurs in approximately half of the *H. pylori* -colonized population. It was estimated that *H. pylori* colonization increases the risk of gastric cancer approximately 10-fold. *H. pylori* was designated a class I carcinogen by the WHO.[(56)]

The risk of development of atrophy and cancer in the presence of *H. pylori* is again related to host and bacterial factors, which influence the severity of the chronic inflammatory response. The lifetime gastric cancer risk among H. pylori-positive subjects is estimated to be approximately1 to 2% in Western countries [57].In the developed world, 60% to 80% of gastric cancers are therefore related to the long-term presence of *H. pylori.*

Gastric MALT (mucosa associated lymphoid tissue) lymphoma

The gastric mucosa does not normally contain lymphoid tissue, but MALT nearly always appears in response to colonization with H. pylori. Nearly all MALT lymphoma patients are *H. pylori* positive,[58] and H. pylori-positive subjects have a significantly increased risk for the development of gastric MALT lymphoma.[59] The exact incidence in H. pylori-positive subjects is unknown, but MALT lymphomas occur in fewer than 1% of H. pylori-positive subjects.[60]

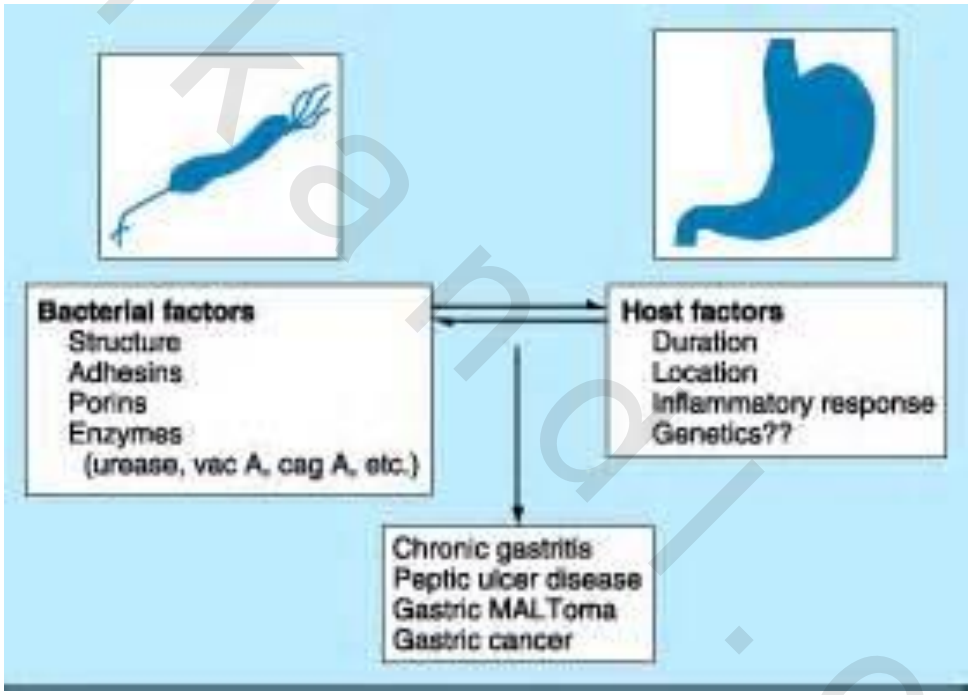

Fig (6): Model representing the role of *H. pylori* and other factor in gastric carcinogenesis,

8-*H. pylori* Virulence Factors

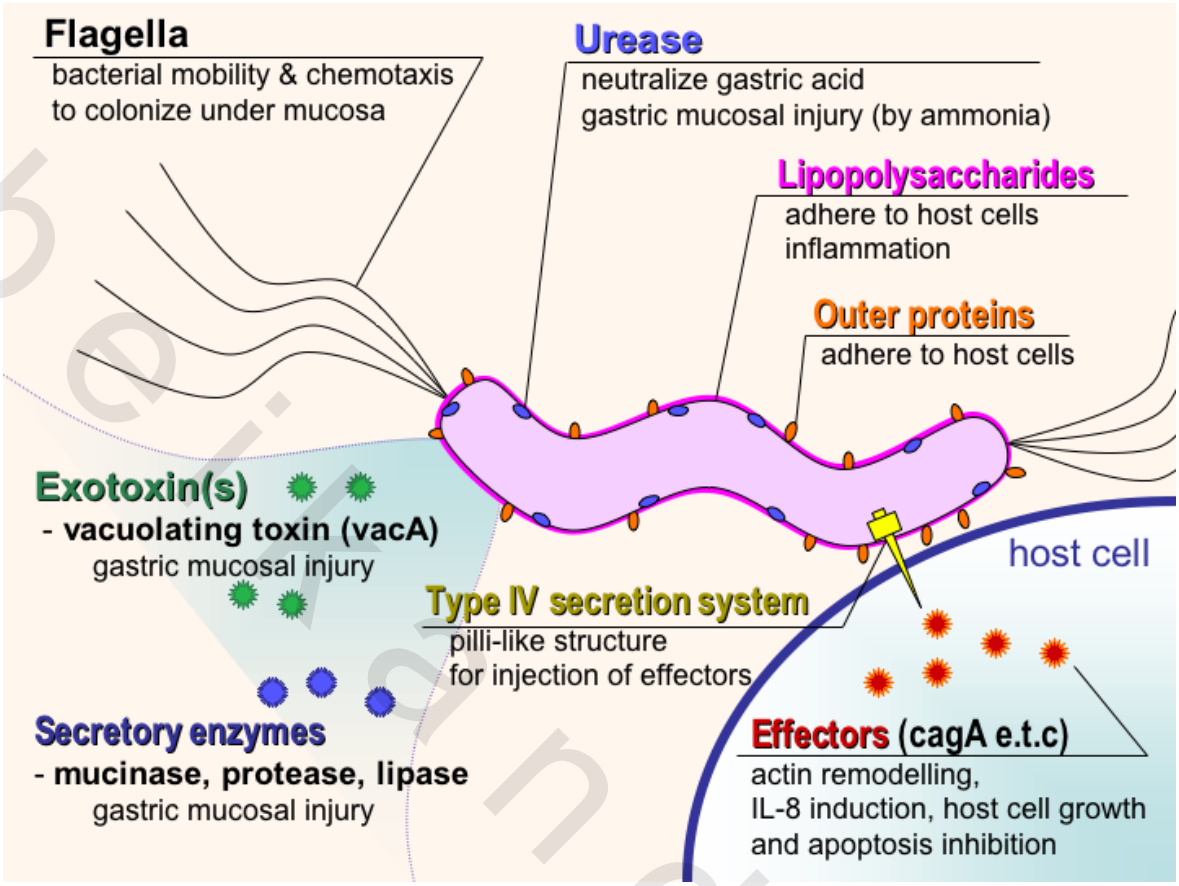

Fig. (7): Components of *Helicobacter pylori* with biological activity: Several of these can have direct effects on host metabolic processes within the cell.[61]

Cag PAI (pathogenesity island)

Early investigations of the differential pathogenic properties of *H. pylori* strains indicated that this increased pathogenicity correlated with the ability virulent strains to induce morphological changes, vacuolization, and successive

Degeneration of in vitro-cultured cells.[62] This activity was then linked to the presence of a protein with a molecular mass of approximately 140 kDa that was named CagA (for "cytotoxin associated gene A").

The *CagA* protein is a highly immunogenic protein encoded by the *cagA* gene.[63] This gene is present in approximately 50 to 70% of *H. pylori* strains[64] and is a marker for the presence of a genomic PAI of about 40 kb that, depending on the strain analyzed, encodes between 27 and 31 proteins.[65] Strains carrying the *cag* PAI (pathogenisty island) are referred to as *CagA*_ strains, as they are commonly identified in patients by their potential to induce significant antibody titers against the *CagA* marker protein. Patients infected with *CagA*_ strains usually have a higher inflammatory response and are significantly more at risk for developing a symptomatic

outcome (peptic ulcer or gastric cancer) in Western populations,[66] though not in Asian populations.[67]

Eighteen of the *cag* PAI-encoded proteins serve as building blocks of a type IV secretion apparatus, which forms a syringe like structure capable of penetrating the gastric epithelial cells and facilitating the translocation of CagA, peptidoglycan, and possibly other bacterial factors into host cells.[68]

Once delivered inside the cell, the CagA protein is phosphorylated at tyrosine residues in EPIYA motifs[68] by Src family kinases.[69] Phosphorylated CagA then interacts with a range of host signaling molecules, such as the tyrosine phosphatase SHP-2 [70].which results in morphological changes in the gastric epithelial cells.[71]

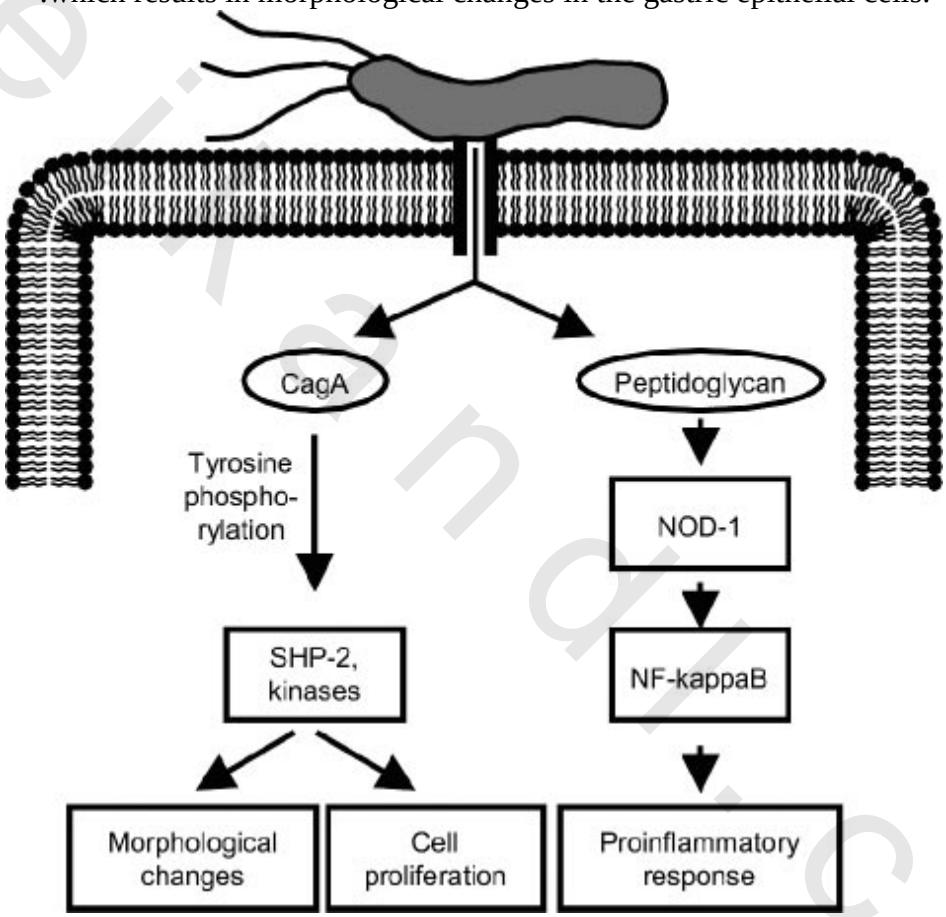

Fig.(8): Schematic representation of the different roles of the Cag type IV secretion system in immune modulation, cell proliferation, and morphological changes.[72]

VacA vacuolating cytotoxin

Approximately 50% of all *H. pylori* strains secrete VacA, a highly immunogenic 95-kDa protein that induces massive vacuolization in epithelial cells in vitro.[72] The VacA protein plays an important role in the pathogenesis of both peptic ulceration and gastric cancer.[73]

Although VacA is not essential for in vitro growth of *H. pylori*, it was reported to significantly contribute to murine gastric colonization by *H. pylori*.[73] The activities

of VacA include membrane channel formation,disruption of endosomal and lysosomal activity, effects on integrin receptor-induced cell signaling, interference with cytoskeleton-dependent cell functions, induction of apoptosis,and immune modulation.(74)

The VacA expression levels differ over time due to the rapid evolution of the bacterium, which seems to be constantly adapting its genetic makeup to facilitate persistent infection.(75) This micro evolution also results in altered toxicity, and the constantly changing toxicity may (in part) explain the constant growing and shrinking of ulcers.(76)

There is a strong correlation between toxin activity and the pathogenicity of *H. pylori*, with the s1/m1 type of VacA being the most virulent in Western populations.(77)

VacA forms pores in epithelial cell membranes, thus inducing the release of urea and anions from the host cells. It also increases transcellular permeability, leading to the release of nutrients and cations.(78) Interestingly, a significant part of the secreted toxin is not released into the environment but remains associated with the outer membrane of *H. pylori* .(79) Upon bacterial contact with host cells, these toxin clusters are transferred to the host cell surface and exert their toxic action.(80)

Acid resistance

One of the striking features of *H. pylori* is the bacterium is not an acidophile. The pH of the gastric mucosa is thought to vary between 4 and 6.5, but occasional acid shocks may occur.(81) *H. pylori* thus requires mechanisms to protect itself from acute acid shocks and mechanisms to grow at pH values around 5.5.(81) The main component of *H. pylori* acid resistance is the urease enzyme, which converts urea into ammonia and carbamate, which spontaneously decomposes into another ammonia molecule and carbon dioxide.(82) Urease activity is present in all *H. pylori* isolates, although the levels of urease activity differ significantly between strains and are dependent on the growth conditions.(83) The ammonia produced by this reaction increases the pH.

Adhesins and outer membrane proteins.

Many bacterial factor mediate the adhesion of *H. pylori* to the gastric epithelium(84).they include:

(i) BabA (HopS)

The 78-kDa BabA protein probably represents the best-characterized *H. pylori* adhesion protein; it is encoded by the *babA* gene. BabA mediates binding to fucosylated Lewis b (Leb) blood group antigens on the human host cells.(85) There are two distinct *babA* alleles, *babA1* and *babA2*, BabA is thought to have a role in the virulence of *H. pylori*, as the *babA2* allele is strongly associated with peptic ulcer disease and gastric adenocarcinoma,(86) but this correlation is controversial.(87) Although the distribution of the *babA* alleles may be associated with more severe disease,(88) the presence of the *babA2* allele is clearly linked to the *vacA* s1 and *cagA* alleles and thus again may not represent an independent disease marker.(89)

(ii) OipA (HopH)

The 34-kDa OipA protein is another member of the Hop protein family that may well serve as an adhesin but was originally identified as a proinflammatory

response- inducing protein.[90] Expression of OipA is strongly associated with increased in vitro and in vivo IL-8 expression, but since the OipA and CagA statuses are linked, this observation requires further study to assess the relative contribution of OipA in gastric inflammation.[91]

(iii) SabA (HopP)

The role of SabA is probably during the chronic inflammatory and atrophic disease stages.[92] SabA also seems to be involved in the binding of the extracellular matrix protein laminin .This may promote an intimate association with the host cell that does not simply assist in evasion of the immune surveillance but might actually allow the bacterium to control the immune response through direct transfer of CagA, VacA, and other virulence factors.[93]

LPS (lipopolysachrides)

The majority of *H. pylori* strains express LPS that contains fucosylated oligosaccharide antigens that are structurally and immunologically closely related to human blood group antigens. These bacterial antigens (Lewis antigens) display marked antigenic variation and are thought to contribute to immune evasion.[94]

The finding that Lewis antigen expression enhances bacterial internalization by epithelial cells [94] suggests that Lewis antigen expression potentially affects the innate immune response.

8-Immune Response

Role of T helper cells in protective immunity

It is now generally accepted that the development of *H. pylori*-induced gastritis or pathology depends predominantly on Th1 cells and Th1 cytokines.[95] Although a Th2-polarized response protect against this specific pathology, this does not necessarily imply that Th2 cells are responsible for protection after immunization.

In fact, Th1-polarized, rather than Th2-polarized, T cells recruit mononuclear cells to the site of infection, resulting in elimination of the bacteria.[95,96] It is now generally accepted that the development of H. pylori-induced gastritis depends on Th1 cells and Th1 cytokines.[97] Although Th2 cells are responsible for protection after immunization.

Immune modulation:

H. pylori infection always results in a strong immune response of the host against the infecting strain, but this response results in clearance of the infection. This adaptive immune response is initiated and maintained by monocytes and Th1 lymphocytes rather than by epithelial cells.

This is because the differentiation of naive T cells into activated Th1 cells requires the presence of IL-12, which is predominantly produced by mononuclear cells. The presence of *H. pylori* in the gastric mucosa is associated with strong IL-12 production[98] and the presence of large numbers of Th1 cells.[99]

Activation of the innate immune response.

Toll-like receptors (TLRs) on epithelial cells recognize and react to semiconserved bacterial products such as flagella (TLR5), peptidoglycan (TLR2), CpG motifs (TLR9), and LPS (TLR4).[100] TLR4-mediated recognition of bacterial LPS is a key activator of the innate immune response in epithelial cells.

H. pylori LPS is a relatively weak inducer. The intracellular peptidoglycan, transferred into the cytoplasm by *cag* PAI (pathogenic island)-mediated contact between the epithelial cell and the bacterium, may be a key activator of the innate response against *H. pylori*.[101]

A member of the recently discovered Nod family. Two members of this protein family, Nod1 (also known as CARD4) and Nod2 (CARD15), are involved in the recognition of bacterial peptidoglycans and seem to act as intracellular (Nod1) and extracellular (Nod2) receptors for gram-negative bacteria in epithelial cells.[102]

Innate immune recognition of *H. pylori* leads to production of proinflammatory cytokines by macrophages, DCs (dendritic cells), mast cells, and gastric epithelial cells.

Role of Regulatory T Cells

The gastric mucosal inflammatory response to *H. pylori* may be regulated in part by regulatory T cells (Tregs) (CD25_CD45RBlo T cells). CD4_/CD25_ Tregs can suppress cytokine production and proliferation of other T cells.[101]

Regulatory immune cells, mostly regulatory FOXP3+CD4+CD25+ T-cells (Treg cells), have been identified as the major regulatory component of the adaptive immune response and involved in H. pylori-related inflammation and bacterial persistence.[101] The functional activity of these cells is either mediated by direct cell-cell contact or by the secretion of the immune-modulating cytokines TNF(tumor necrotizing factor)-β1 and IL-10. Based on the differentiation process, Treg cells comprise various lineages that differ in the expression of cell surface marker and pattern of secreted cytokines.

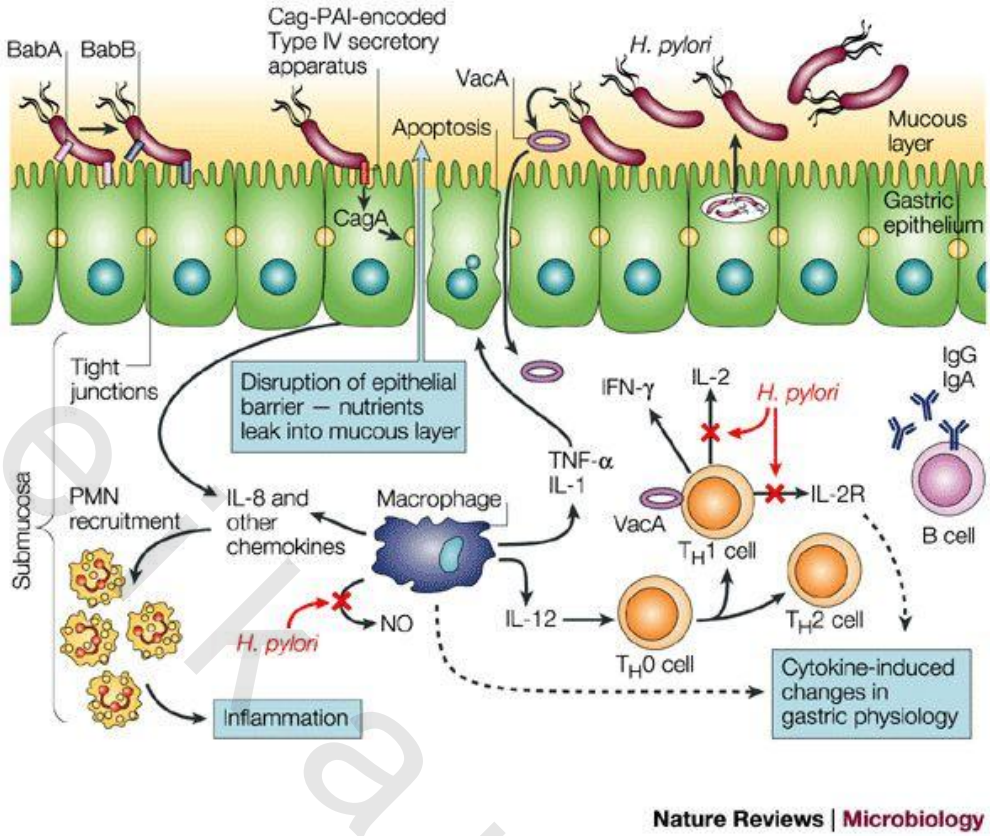

Fig. (9): Schematic demonstrating how dendritic cells may bridge the innate and adaptice immune response directed against *H. pylori* within the gastric mucosa. Dendritic cells can penetrate the epithelial barrier in vivo and sample *H. pylori* antigens directly. Dendritic cells, in turn, activate T cells in different ways, being capable of inducing either a Th1, Th2/regulatory T cell (Treg), or a Th17 response by generation of interleukin (IL)-12, IL-10, or IL-23, respectively. Direct interactions between *H. pylori* and gastric epithelial cells or *H. pylori* constituents such as urease can also activate polymorphonuclear (PMN) cells and/or macrophages, which further amplifies the T-cell response to this pathogen. Copyright © 2012 the American Physiological Society

Interactions of *H. pylori* with Neutrophils

Neutrophils are recruited when *H. pylori* initially colonizes the human stomach,[102] persistent *H. pylori* infection is characterized by infiltration of neutrophils.[103] Several specific *H. pylori* factors are known to interact with neutrophils and modulate their function.

Interactions of *H. pylori* with Mast Cells

In vitro experiments indicate that whole *H. pylori* bacteria and various *H. pylori* components can activate mast cells.[105] One *H. pylori* factor that can activate mast cells is VacA. VacA can induce mast cell chemotaxis and can stimulate mast cell expression of multiple proinflammatory cytokines, including IL-1, TNF, IL-6, IL-13, and IL-10.[106]

Interactions of *H. pylori* with Macrophages

Contact between macrophages and intact *H. pylori* bacteria or *H. pylori* components results in macrophage activation and the secretion of numerous cytokines and chemokines .Macrophages recognize *H. pylori* LPS via TLR4[107] and can also be

activated by *H. pylori* proteins, including urease.[108] Macrophage recognition of intact *H. pylori* can be mediated by TLR2 or TLR4.[109]

Interactions of *H. pylori* with Dendritic Cells

H. pylori also stimulates dendritic cell expression of multiple cytokines, including IL-6, IL-8, IL-10, and IL-12.[110]

Interactions of *H. pylori* with T Lymphocytes

In vitro experiments indicate that live *H. pylori* or *H. pylori* products can interfere with multiple functions of T lymphocytes.[111] One report indicated that *H. pylori* can have proapoptotic effects on T cells,[112] but most of the observed effects occur in the absence of cell death. Coincubation of *H. pylori* with T cells results in diminished expression of IL-2 and IL-2 receptor (CD25), inhibition of activation-induced proliferation, and cell cycle arrest.[113,114]

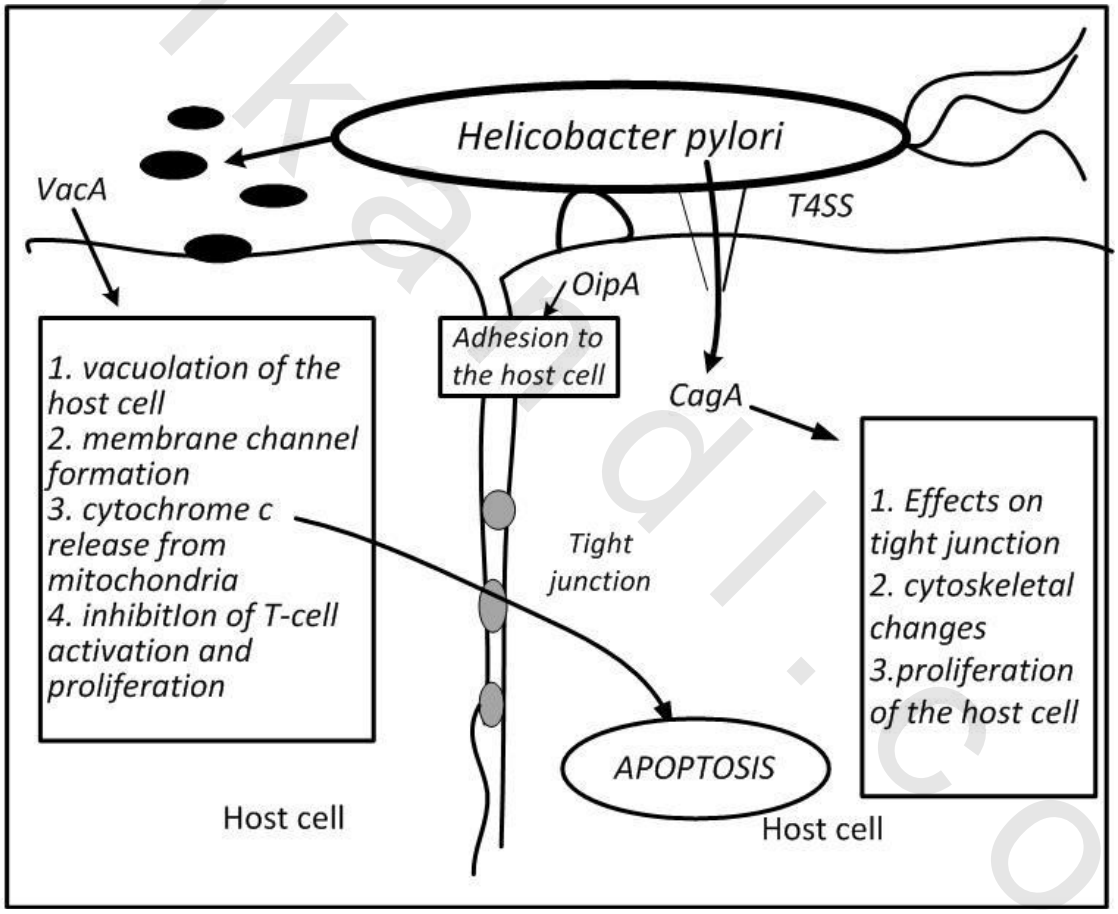

Fig. (10): Illustrates how the virulence factors attack the host cell

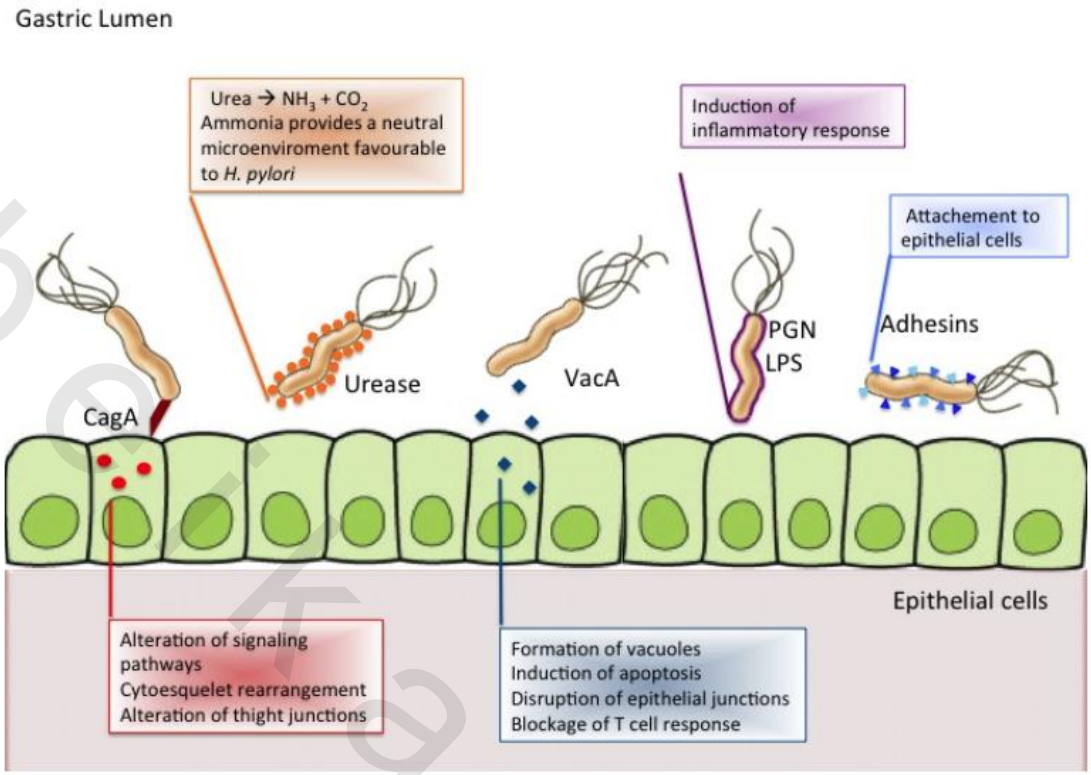

Fig. (11): The effect of Virulence factors on the mucosal layer of the stomach

9-The Relationship between *H. pylori* and hepatitis C virus

Cammarota *et al*. reported a high prevalence of *H. pylori* and hepatitis C virus (HCV) in the stomach of patients with HCV infection. In these subjects, the presence of both HCV and *H. pylori* in the gastric mucosa was significantly associated with marked or moderate inflammatory infiltration Oligoclonal immunoglobulin H-gene rearrangements were detected in patients who harbored both *H. pylori* and HCV in their stomach. It is suggested that HCV and *H. pylori* cooperate to induce chronic lymphocytic inflammation.[115] Some researchers have found a high seroprevalence of antibodies to *H. pylori* in patients with HCV-positive liver diseases.[115]

Chronic hepatitis is an inflammatory process and as with any other chronic inflammation is characterized by a rise in pro-inflammatory cytokines like IL1, IL6, TNF, etc..[116] Viruses, including HCV, are capable of inducing limited inflammation,[117] contrary to bacteria like *H. pylori*, which are potent inducers of the inflammatory cascade.[118] It has been shown that *H. pylori* can cause proto-oncogene activation that is likely to the key step in the pathway of *H. pylori* induced neoplasia.[119]

The currently available information is that *H. pylori* having a definite role in the evolution of CLD and/or HCC. But *H. pylori* eradication therapy has shown to be beneficial in patients with CLD and/or HCC who are co-infected with H. pylori. It is clear that there is increasing association of *H. pylori* with CLD.

Moreover, both *H. pylori* and HCV have pathogenic influence on gastric and liver epithelium, including inducing the risk of malignant transformation. This definitely warrants further studies. An article from an Egyptian group, published in this issue provokes thoughts and provides intellectual inputs in this direction. It is perhaps the first step in the right direction to an unexplored arena, still not fully known to us.

Hematological malignancies

Hematological malignancies arise when the processes of proliferation or apoptosis are corrupted in blood cells. The leukemia, lymphoma and multiple myeloma are an interrelated spectrum of malignancies of the myeloid and lymphoid systems.(120)

The myeloid cell line normally produces granulocytes, erythrocytes, thrombocytes, macrophages and mast cells; the lymphoid cell line produces B, T, NK and plasma cells. Lymphoma, lymphocytic leukemia, and myeloma are from the lymphoid line, while acute and chronic myelogenous leukemia, myelodysplastic syndromes and myeloproliferative diseases are myeloid in origin.(120)

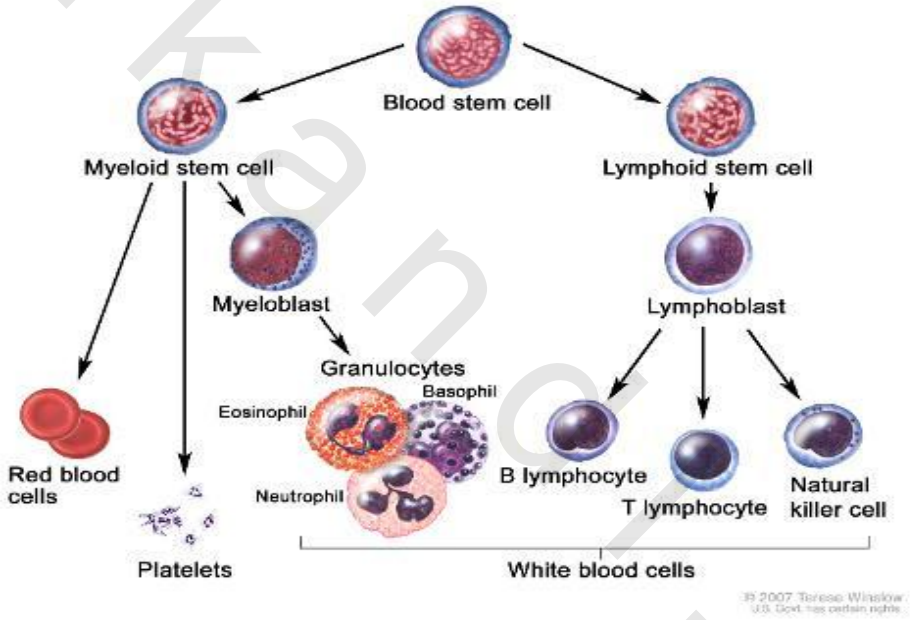

Fig. (12): Development of different blood cells from hematopoietic stem cell to mature cells. (121)

Classification and incidence

Hematological malignancies represent 9 % of new cases of cancer in NSW. Also, they account for 9.5% of new cancer diagnoses in the United States.(122)

Hematological malignancies are defined and distinguished from one another essentially according to 4 parameters: clinical features, microscopic morphology, immunophenotype and molecular/genetic features.(123,124)

Hematologic malignancies are broadly divided into myeloid neoplasms, lymphoid neoplasms (leukemias and lymphomas), lymphoproliferative disorders, and histiocytic/dendritic cell neoplasms.(123,124)

The WHO Classification of Hematologic Malignancies:

I-Myeloid Neoplasms

A. Acute myeloid leukemias/ myeloid sarcoma (AML)

B. Other myeloid neoplasms

i. Myeloproliferative neoplasms
   1. Chronic leukemias with mature cells in peripheral blood and bone marrow
   2. High cell counts in peripheral blood
   3. Variable propensity for transformation to acute myeloid leukemia

ii. Myelodysplastic syndromes
   1. One or more cytopenias in the peripheral blood
   2. Bone marrow is hypercellular and cells look abnormal (dysplastic)
   3. Variable propensity for transformation to acute myeloid leukemia

II. Lymphoid Neoplasms

A. Non-Hodgkin lymphomas (NHL)

i. Precursor lymphoid neoplasms
   1. May arise as leukemias or lymphomas
   2. Composed of immature B or T cells
      a. B lymphoblastic leukemia/lymphoma
         i. More commonly presents as acute leukemia
         ii. Immunophenotyping - CD19, CD22, CD10, TdT, CD34
         iii. Recurrent genetic abnormalities common and important for prognosis and treatment stratification
      b. T lymphoblastic leukemia/lymphoma
         i. More commonly presents as lymphoma, mediastinal mass common
         ii. Immunophenotyping - CD3 (cytoplasmic), CD2, CD5, CD7, usually either CD4/CD8 double positive or double negative, CD1a, TdT

ii. Mature B cell lymphomas
   1. Small B cell lymphomas (most are low grade except mantle cell)
      a. Chronic lymphocytic leukemia/small lymphocytic lymphoma
         i. Usually presents as a leukemia
         ii. In lymph nodes - vaguely nodular pattern with proliferation centers
         iii. Immunophenotype: CD19, CD20 (dim), CD5, CD23
         iv. Some risk of transformation to diffuse large B cell lymphoma
      b. Follicular lymphoma
         i. Usually presents in lymph nodes with widespread disease at presentation
         ii. Nodular proliferation of monotonous crowded abnormal germinal centers
         iii. Immunophenotype: CD19, CD20, CD10, BCL-2, BCL-6
         v. Some risk of transformation to diffuse large B cell lymphoma
         vi. Genetics: t(14;18) IgH/BCL2
      c. Mantle cell lymphoma
         i. Usually present in lymph nodes with widespread disease at presentation
         ii. Diffuse sheets of monotonous small lymphocytes
         iii. Unlike other small B cell lymphomas, it behaves aggressively clinically
         iv. Immunophenotype: CD19, CD20, CD5, Cyclin D1
         v. Genetics: t(11;14) IgH/CCND1

d. Marginal zone lymphoma
   i. 3 settings - nodal, splenic, or MALT
   iii. Diffuse or nodular sheets of small B cells with a lot of clear cytoplasm; colonize normal bystander germinal centers
   iv. Immunophenotype: CD19, CD20 (negative for CD5, CD10, and CD23)
   v. Genetics: variable - t(11;18) API/MALT1 seen in some cases of MALT lymphomas of the stomach

2. More aggressive B cell lymphomas
   a. Diffuse large B cell lymphoma
      i. Usually involves lymph nodes, but commonly involves extranodal sites
      ii. Recently subdivided into various subtypes based on clinical setting, immunophenotype, associations with viruses, morphology
      iii. Sheets of large (larger than a histiocyte nucleus) atypical lymphoid cells
      iv. Immunophenotype: CD19, CD20
      v. May be de novo or arise out of a lower grade lymphoma
   b. Burkitt lymphoma
      i. Extranodal involvement very common (especially GI in the Western world)
      ii. Sheets of medium size lymphoid cells with a "starry sky"
      iii. Some cases are EBV related
      iv. Very aggressive and very rapid doubling time
      vi. Immunophenotype: CD19, CD20, CD10, BCL6
      vii. Genetics: t(8;14) IgH/CMYC most commonly or t(2;8) or t(8;22)

3. Plasma cell neoplasms
   a. May be marrow based (myeloma) or solitary lesion (plasmacytoma)
   b. Sheets of clonal plasma cells
   c. Immunophenotype: CD19, CD38, CD138, CD20+/-, kappa or lambda light chain, CD56

iii. Mature T/NK cell lymphomas

1. Peripheral T cell lymphoma, NOS
   a. Most commonly nodal, but may be extranodal
   b. Aggressive clinical course
   c. Diffuse sheets of small, medium, and large atypical lymphoid cells admixed with histiocytes and eosinophils
   d. Immunophenotype: CD3, CD2, CD5, CD7, CD4 or CD8

2. Anaplastic large cell lymphoma
   a. Most cases are ALK positive
   b. Sinusoidal infiltrate of large atypical cells with reniform or horseshoe shaped nuclei
   c. Immunophenotype: CD3, CD2, CD4, CD16, EMA
   d. ALK positive cases have t(2;5) NPM/ALK translocation (most commonly)

B. Hodgkin lymphomas (HL)

i. Characterized by contiguous spread
ii. Neoplastic cells are the Reed-Sternberg cells and Hodgkin cells

iii. Majority of cells seen are reactive lymphocytes, histiocytes, granulocytes, and eosinophils with only relatively few neoplastic cells
iv. Classified as classical or nodular lymphocyte predominant based on immunophenotype of the neoplastic cells
    1. Nodular lymphocyte predominant: CD45, CD20, PAX5 positive and negative for CD15 and CD16
    2. Classical: CD15, CD16, and PAX5 positive (CD20 +/-) and negative for CD45

III. Histiocytic and Dendritic cell neoplasms

IV. Hematologic neoplasms with a propensity for bony involvement (forming tumor masses)

A. Plasma cell neoplasms (plasmacytoma, plasma cell myeloma)
B. Diffuse large B cell lymphoma
C. Langerhans cell histiocytosis
D. Less common
    i. Anaplastic large cell lymphoma
    ii. Myeloid sarcoma
    iii. Burkitt lymphoma
    iv. Hodgkin lymphoma
    v. Plasmablastic lymphoma[123,124]

So, a lymphoma is a lymphoid malignancy that involves lymph nodes and/or other extramedullary sites. They are broadly classified as being Hodgkin or non-Hodgkin and are treated with very different therapies. Non-Hodgkin lymphomas may be immature (lymphoblastic), although the vast majority have a mature B,T, or NK cell phenotype.[123,124]

Leukemia is any myeloid or lymphoid malignancy that largely involves the peripheral blood and bone marrow. They may be classified as acute or chronic, depending maturity of the cell of origin (acute = blasts and chronic = more mature cells), which also tends to correlate with the clinical acuity (acute = weeks and chronic = years).[123,124]

So, leukemia is divided into:

1-Acute leukemia:

- Acute lymphoblastic leukemia (ALL)
- Acute myelogenous leukemia (AML)

2-Chronic leukemia:

- Chronic myelogenous leukemia (CML)
- Chronic lymphocytic leukemia (CLL)[123,124]

Acute leukaemia

1-Acute myeloid leukemia (AML)

Acute myeloid leukemia (AML), also known as acute myelogenous leukemia or acute nonlymphocytic leukemia (ANLL), is a cancer of the myeloid line of blood cells,

characterized by the rapid growth of abnormal white blood cells that accumulate in the bone marrow and interfere with the production of normal blood cells. [125]

The malignant cell in AML is the myeloblast. In normal hematopoiesis, the myeloblast is an immature precursor of myeloid white blood cells; a normal myeloblast will gradually mature into a mature white blood cell. In AML, though, a single myeloblast accumulates genetic changes which "freeze" the cell in its immature state and prevent differentiation.[126]

Such a mutation alone does not cause leukemia; however, when such a "differentiation arrest" is combined with other mutations which disrupt genes controlling proliferation, the result is the uncontrolled growth of an immature clone of cells, leading to the clinical entity of AML.[127]

The clinical signs and symptoms of AML result from the growth of leukemic clone cells, which tends to displace or interfere with the development of normal blood cells in the bone marrow. This leads to neutropenia, anemia, and thrombocytopenia. The symptoms of AML are, in turn, often due to the low numbers of these normal blood elements.[128]

In rare cases, patients can develop a chloroma, or solid tumor of leukemic cells outside the bone marrow, which can cause various symptoms depending on its location.[128]

Acute myeloid leukemia (AML) is one of the most common types of leukemia among adults. This type of cancer is rare under age 40.[129]

Classification of AML

There are two main classifications of AML:

- The initial FAB classification
- The modern WHO classification.

The French-American-British (FAB) classification system divides AML into eight subtypes, M0 through to M7, based on the type of cell from which the leukemia developed and its degree of maturity. This is done by examining the appearance of the malignant cells with light microscopy and/or by using cytogenetics to characterize any underlying chromosomal abnormalities. The subtypes have varying prognoses and responses to therapy.[130]

Table (II): FAB classification of acute myeloid leukemias [130]

Subclass	Description
M0	Acute non-differentiated leukemia – immature blast cells with minimal differentiation
M1	Acute myeloblastic leukemia without maturation – immature blast cells without signs of myeloid differentiation
M2	Acute myeloblastic leukemia with granulocytic maturation
M3	Promyelocytic or acute promyelocytic leukemia (APL)
M4	Acute myelomonocytic leukemia
M4eo	Myelomonocytic leukemia with bone marrow eosinophilia
M5	M5a – acute monocytic leukemia without maturation M5b – acute monocytic leukemia with partial maturation
M6	Acute erythromyelosis
M7	Acute megakaryoblastic leukemia

The WHO classification of acute myeloid leukemia was developed considering the FAB system, but it is more convenient for clinical application because it takes into account the most significant prognostic signs of the disease. Mutations described for the first group are located in the genes highly sensitive to damage caused by some chemical preparations and, thus, can be involved in the appearance of the third group AML.[131]

Table (III): Subspecies of acute myeloid leukemias according to the WHO classification.[131]

Group	Subspecies	Description
IL with specific ietic changes	– AML with translocation t(8;21)(q22;q22), (AML1/ETO); – AML with blood marrow eosinophilia and inversions [inv(16)(p13q22) or t(16;16)(p13;q22); CBFB/MYH11]; – AML with translocations [t(15;17)(q22;q12) (PML/RARα)]; – AML with mutation 11q23 (MLL)	Patients usually have high level of remissions and show good response to therapy. Prognosis is better than for other subspecies of AML. It is usually observed in children and patients younger than 20 years. The subtypes are rather easily identified morphologically
IL with dysplasia several hematoetic stem cells	– develops from MDS or MDS/MPD; – there are no preceding MDS or MDS/MPD, but dysplasia of more than 50% of cells of two or more myeloid stem cells	The group is characterized by unfavorable prognosis, and probability of this subspecies increases with age
IL and MDS ociated with preus treatment	– alkylating agents/radiation (arises 4-7 years after exposure, is characterized by mutations affecting chromosomes 5 and 7); – inhibitor of topoisomerase II (arises 2-3 years after exposure, is specified by mutations: 11q23, 21q22, inv(16)(p13q22), t(15;17)(q22;q12))	This AML subspecies involves patients treated by chemo- and/or radiotherapy after which AML or MPD appeared. In these leukemias chromosomes can carry specific changes often associated with worse prognosis
IL not corrending to signs of ed subtypes	– AML without maturation; – AML with minimal differentiation; – AML with maturation; – acute myelomonocytic leukemia; – acute monocytic leukemia; – acute erythroid leukemia; – acute megakaryoblastic leukemia; – acute basophilic leukemia; – acute panmyelosis (hyperplasia of all bone marrow stem cells) with myelofibrosis; – osteoblastosarcoma	Includes AML subspecies not involved in the above-listed ones or which cannot be analyzed genetically

Risk factors

- Increasing age. The risk of acute myelogenous leukemia increases with age.
- Sex. Men are more likely to develop acute myelogenous leukemia than are women.
- Previous cancer treatment. People who've had certain types of chemotherapy and radiation therapy may have a greater risk of developing AML.
- Exposure to radiation. People exposed to very high levels of radiation, such as survivors of a nuclear reactor accident, have an increased risk of developing AML.
- Dangerous chemical exposure. Exposure to certain chemicals, such as benzene, is linked to greater risk of AML.
- Smoking. AML is linked to cigarette smoke, which contains benzene and other known cancer-causing chemicals.
- Other blood disorders. People who've had another blood disorder, such as myelodysplasia, polycythemia vera or thrombocythemia, are at greater risk of developing AML.
- Genetic disorders. Certain genetic disorders, such as Down syndrome, are associated with an increased risk of AML. [131-133]

Acute lymphoblastic leukemia (ALL)

Acute lymphoblastic leukemia (ALL) is a form of leukemia characterized by excess lymphoblasts. Malignant, immature white blood cells continuously multiply and are overproduced in the bone marrow.(134)

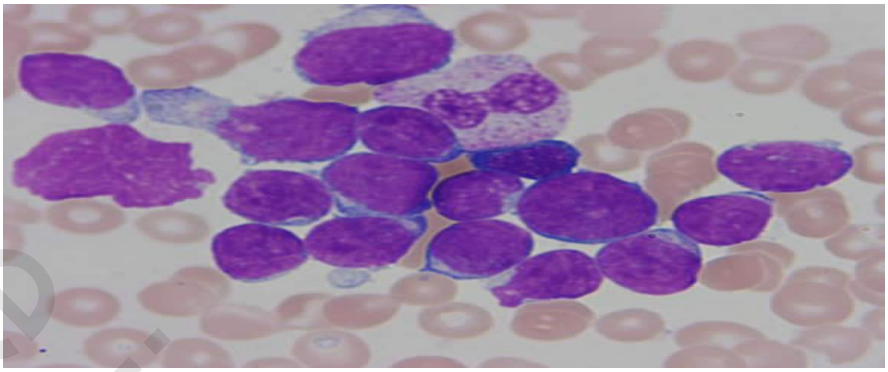

Fig. (13): The large purple cells below are ALL blast cells. (134)

Causes

The causes of the disease are not known, but researchers believe that ALL develops from a combination of genetic, biologic, and environmental factors.(135)

Genetic Translocations

Up to 65% of leukemias contain genetic rearrangements, called translocations, in which some of the genetic material (genes) on a chromosome may be shuffled or swapped between a pair of chromosomes.(135)

- The most common genetic translocation in ALL is the Philadelphia (Ph) chromosome where DNA is swapped between chromosomes 9 and 22 [t (9:22)]. It occurs in about 20 - 16% of adults and 3 - 5% of children with ALL.(16)
- Another common translocation in ALL is t (12; 21), which is referred to as TEL-AML1 fusion. It occurs in about 20% of patients with ALL. Researchers believe that this translocation may occur during fetal development in some patients.(135)

Risk Factors

Age

ALL in Children. ALL is the most common type of cancer diagnosed in children. ALL accounts for about 75% of cases of childhood leukemia. Each year, about 3,600 American children and adolescents are diagnosed with ALL. ALL can strike children of all ages, but is most likely to occur when children are 2 - 4 years of age. It is slightly more common in boys than in girls.(136)

ALL in Adults. ALL is the least common type of leukemia among adults. About 1 in 3 cases of ALL occur in adults.(136)

Race and Ethnicity

Caucasian and Hispanic children have a higher risk for ALL than African-American children.(136)

Hereditary Disorders

ALL does not appear to run in families. But certain inherited genetic disorders may increase risk. For example, children with Down syndrome have a 20-times greater risk of developing ALL than the general population. Other rare genetic disorders associated with increased risk include Klinefelter syndrome, Bloom syndrome, Fanconi anemia, ataxia-telangiectasia, neurofibromatosis, Shwachman syndrome, IgA deficiency, and congenital X-linked agammaglobulinemia.(137)

Radiation and Chemical Exposure

Previous cancer treatment with high doses of radiation or chemotherapy can increase the risk for developing ALL. Prenatal exposure to x-rays may also increase risk in children.(135)

Classification

The FAB classification

Subtyping of the various forms of ALL used to be done according to the French-American-British (FAB) classification, which was used for all acute leukemias (including acute myelogenous leukemia, AML).

- ALL-L1: small uniform cells
- ALL-L2: large varied cells
- ALL-L3:large varied cells with vacuoles (bubble-like features).(130)

Each subtype is then further classified by determining the surface markers of the abnormal lymphocytes, called immunophenotyping. There are 2 main immunologic types: pre-B cell and pre-T cell. The mature B-cell ALL (L3) is now classified as Burkitt's lymphoma/leukemia. Subtyping helps determine the prognosis and most appropriate treatment in treating ALL.(130)

WHO proposed classification of acute lymphoblastic leukemia

The recent WHO International panel on ALL recommends that the FAB classification be abandoned, since the morphological classification has no clinical or prognostic relevance. It instead advocates the use of the immunophenotypic classification mentioned below.

1- Acute lymphoblastic leukemia/lymphoma Synonyms:Former Fab L1/L2

- Precursor B acute lymphoblastic leukemia/lymphoma. Cytogenetic subtypes:
    - t(12;21)(p12,q22) TEL/AML-1
    - t(1;19)(q23;p13) PBX/E2A
    - t(9;22)(q34;q11) ABL/BCR
    - T(V,11)(V;q23) V/MLL
- Precursor T acute lymphoblastic leukemia/lymphoma

2- Burkitt's leukemia/lymphoma Synonyms:Former FAB L3

3- Biphenotypic acute leukemia.(123)

10- Suggested mechanism of how *Helicobacter pylori* infection causes leukemia and malignant lymphoma?

The question of whether *H. pylori* could play a role in the development of malignant lymphoma and leukemia remains controversial.

Original Articles *H. pylori* infection is associated with an increased risk of gastric lymphomas, both MALT and DLBCL, and of splenic MZL. No other histologies, nor other sites of lymphoma, were associated with *H. pylori* infection. In addition to corroborating established clinical and pathologic knowledge on gastric lymphoma,[138] The two common malignant neoplasms that arise in the stomach are adenocarcinoma and lymphoma of gastric mucosal associated lymphoid tissue (MALT). While the incidence of gastric carcinoma has declined in many developing countries, it is still exceedingly prevalent in most of the developing world, and is the second leading cause of cancer-related death worldwide.[139] Most gastric cancers are still detected at an advanced stage. Consequently, the prognosis of this disease remains very poor, even after extensive surgery and adjuvant therapy.[139] Gastric MALT lymphoma is considerably less common than gastric carcinoma, accounting for 3% of all gastric tumors.[140]

Both gastric carcinomas and MALT lymphomas have long been recognized to occur on a background of chronic gastric inflammation. For the past two decades it has been evident that the usual cause of this gastritis is persistent infection by the gram-negative micro-aerophilic bacterium *H.pylori*. Approximately 70% of all gastric cancer cases worldwide are directly attributable to prior *H. pylori* infection,[141] as are the majority of gastric MALT lymphomas.[140]

Currently, about half of the world's population is infected by *H. pylori*, with rates in the developed world in the order of 70%.[142] Gastric colonization by *H. pylori* is usually asymptomatic and although about 20% of the infected population progress to some extent down the "Correa" pathway of pre-neoplastic changes over several decades, gastric neoplasms develop in fewer than 2%.[143] Gastric lymphoma is an even rarer consequence of *H. pylori* infection, occurring in fewer than 1% of those who are infected. However, based upon the available epidemiological evidence, the World Health Organization's International Agency for Research on Cancer classified*H. pylori* as a group I or definite carcinogen (the only bacterium to be thus classified) in 1994.[144] Since that time evidence linking *H. pylori* to gastric cancer has continued to accumulate and strengthen.

Numerous epidemiological, animal and experimental studies support a positive association between chronic *H. pylori* infection and the development of distal gastric cancer and gastric MALT lymphoma. However, the molecular cellular events responsible for the promotion of these gastric malignancies by *H. pylori* remain poorly defined. Current evidence suggests that the bacterium itself has carcinogenic effects and that the inflammatory response to *H. pylori*, which is highly variable, can contribute to lowering the threshold for gastric cancer development.

Among the molecular mechanisms that are thought to be important in *H. pylori*-induced gastric carcinogenesis are the induction of oxidative and nitrosative stress with consequent cellular and DNA damage followed by cycles of repair. Ultimately, as

antioxidant defenses and damage- repair responses are overwhelmed and depleted, genetic errors that arise under the pressure of accelerated gastric epithelial turnover may accumulate to the point at which neoplastic transformation is inevitable. Many of these events occurring in the chronically inflamed gastric mucosa are common to other inflammation-associated malignancies, while some are unique to *H. pylori* infection.[145] In this review we shall discuss *H. pylori* as an agent in gastric carcinogenesis and consider the mechanisms responsible for its pathogenesis. Like many other cancer-inducing infections, *H. pylori* does not promote cancer universally. Indeed, while over 50% of the world's population is infected with *H. pylori*, only 2% progress to gastric cancer, and even fewer develop a MALT lymphoma. Given this variable risk of malignancy following *H. pylori* infection, what are the critical factors or co-factors involved in determining which individuals infected by *H. pylori* will undergo *H. pylori*-induced gastric transformation? Some of this variability in outcome can be correlated with bacterial strain specificity, host genetic susceptibility, and the type of immune response elicited in the infected host. *H. pylori* contains virulence factors such as the *cag* pathogenicity island that induce changes in cellular morphology *in vitro* and alters signaling pathways and gene expression patterns; other putative virulence factors such as the BabA2 adhesin and the VacA exotoxin have not been consistently correlated with cancer susceptibility. Host polymorphisms in cytokine and cytokine receptor genes such as IL-1B, IL-1RB, TNF and IL-10 that regulate inflammatory response may explain why certain individuals within susceptible populations develop worse H. pylori-induced gastric pathology, as well as illustrate the important role that the host's inflammatory and immune responses play in the pathophysiology of *H. pylori* infection.

What makes *H. pylori* different from some other cancer causing infectious organisms, particularly certain viruses, is the fact that this extracellular bacterium can be viewed as predominantly an *indirect* carcinogen,[146] promoting neoplastic changes through the associated chronic inflammatory response. Key elements of the carcinogenic gastric inflammatory reaction to *H. pylori* include oxidative stress, gastric immune cell accumulation and pro-inflammatory cytokine production leading to increased epithelial turnover and, over time, cellular transformation. The demonstration of the recruitment of bone marrow-derived cells to the tumor microenvironment during gastric carcinogenesis offers additional directions for investigating the indirect mechanisms of cancer causation by *H. pylori*.

Gastric MALT lymphoma is a rare type of non-Hodgkin lymphoma that is characterized by the slow multiplication of B lymphocytes, a type of immune cell, in the stomach lining. This cancer represents approximately 12 percent of the extranodal (outside of lymph nodes) non-Hodgkin lymphoma that occurs among men and approximately 18 percent of extranodal non-Hodgkin lymphoma among women.[147] During the period 1999–2003, the annual incidence of gastric MALT lymphoma in the United States was about one case for every 100,000 persons in the population.

Normally, the lining of the stomach lacks lymphoid (immune system) tissue, but development of this tissue is often stimulated in response to colonization of the lining by *H. pylori*.[148] Only in rare cases does this tissue give rise to MALT lymphoma. However, nearly all patients with gastric MALT lymphoma show signs of *H. pylori*

infection, and the risk of developing this tumor is more than six times higher in infected people than in uninfected people.[149,150] Indirect antigenic stimulation by *H. pylori*-specific T cells is implicated in the development of low-grade gastric lymphoma of mucosa-associated lymphoid tissue (MALT), however, the role of direct antigen stimulation is unknown. To study the role of direct antigen stimulation in MALT lymphomagenesis and its relationship with the pathogenesis of distinct pathological lesions, which represent different stages of the tumour progression, we cloned and sequenced the rearranged immunoglobulin (Ig) heavy chain gene in three low-grade (two from the lung, one from the stomach) and one high-grade (from the stomach) cases. In the low-grade gastric case, we studied the Ig sequence in primary as well as its disseminated and recurrent tumours. In the high-grade gastric case, we analysed the Ig sequence intumour cell populations microdissected from the residual diffuse low-grade lesions, diffuse high-grade areas from follicles colonized by high-grade blasts. Compared with the published germline sequences, the heavy chain variable (VH) genes of three MALT lymphomas, in which the putative germline was identified, contained frequent somatic mutations, showing a much higher ratio of replacement/silent mutations in the complementarity determining regions (CDRs) than the framework regions (FRs). Ongoing mutation as indicated by intraclonal variation of the Ig sequence clearly existed in low-gradetumour including its dissemination and recurrence, but was not evident in high-grade tumour cell populations including those microdissected from independent colonized follicles. In addition, the germlines of VH genes used by the three MALT lymphomas are frequently found in autoreactive antibodies. Our results suggest that MALT lymphoma derives from postgerminal centre memory B cells, possibly autoreactive B cell clones, and that direct antigen stimulation may play an important role in the clonal expansion of low-grade MALT lymphoma.

Table (IV): Statistics of chronic and acute lymphocytic leukemia in Northern of Africa (Egypt)

Chronic lymphocytic leukemia in Northern Africa (Extrapolated Statistics)		
Egypt	2,291	76,117,4212
Acute lymphocytic leukemia in Northern Africa (Extrapolated Statistics)		
Egypt	1,071	76,117,4212

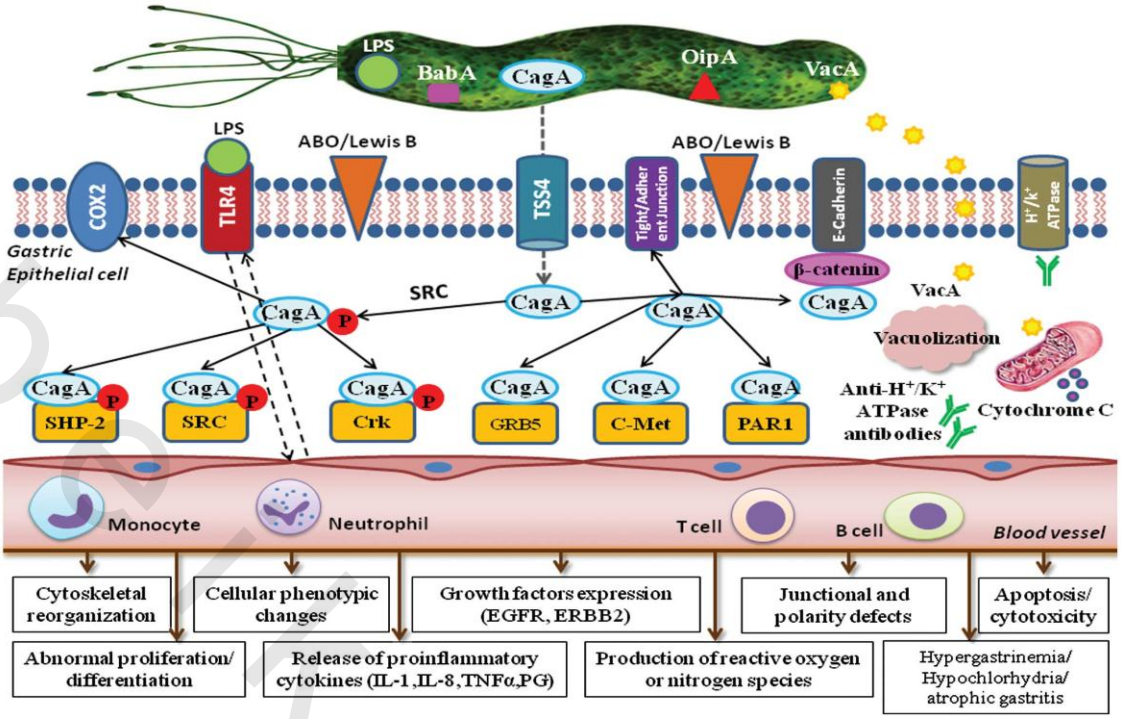

Fig. (14): Illustrating the mechanism of *H. pylori* in malignancy

11- Diagnostic tests for *H. pylori* infection:

H. pylori infection can be diagnosed either directly (by demonstration of the organism) or indirectly (measurement of urease activity, antibody detection). Both invasive and non-invasive diagnostic techniques can be used. All of the invasive methods are based on endoscopy and the acquisition of biopsy specimens. The various tests have high sensitivity and specificity[151-153] but false-positive and false-negative results remain possible. The former may be due to bacterial colonization of the mouth, pharynx, or stomach, while the latter may be seen in cases of acute upper gastrointestinal bleeding, or when the bacterial colonization is of low density in the aftermath of partial gastric resection or suppressive treatment for *H. pylori*.[154]

A. Invasive methods:

H. pylori can be readily detected at endoscopy by histology, culture or urease tests, but all biopsy-based methods are liable to sampling error, because infection is patchy.[155] Up to 14% of patients with *H. pylori* will not have antral infection but will have *H. pylori* elsewhere in the stomach, especially if there is gastric atrophy, intestinal metaplasia or bile reflux. In addition after partially effective eradication therapy low levels of recurrent infection can be easily missed by biopsy leading to overestimates of the efficacy of eradication therapy. For these reasons consensus guidelines recommend taking multiple biopsies from the antrum and corpus for both histology and one other method (either culture or urease testing).[156,157]

1. Histology:

Although *H. pylori* may be recognized on H&E stained sections alone, special supplementary stains (e.g. Giemsa, Gimenez) are always needed to detect low levels of infection and show the characteristic morphology of *H. pylori*[154]. Histology also provides an historical record: sections (or additional sections) can always be

reexamined and atrophy or intestinal metaplasia assessed. Additional biopsies from other parts of the stomach can be retained in formalin and only processed if antral histology is inconclusive.[156]

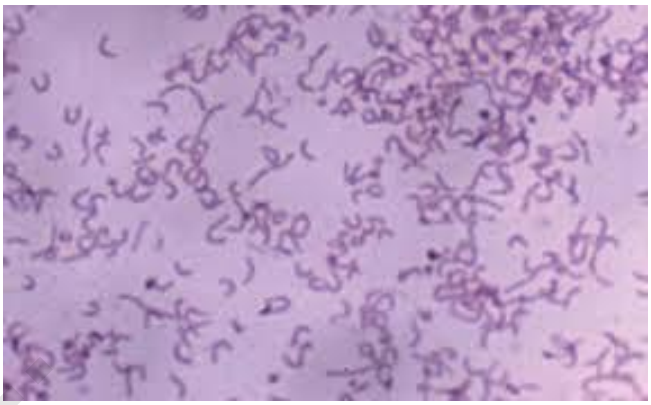

Fig. (15): The gram stain showing the comma shaped morphology of *H.pylori*

2. Culture:

Microbiological isolation is the theoretical 'gold standard' for identifying any bacterial infection, however, for *H. pylori* culture can be unreliable, with risks of overgrowth or contamination making it the least sensitive method of detection and the least readily available of endoscopic methods.

Culture and antibiotic sensitivity testing is also necessary because of the prevalence of multiple antibiotic resistant strains,[157] such information might be useful to guide therapy, especially for individual patients whose infection has not been cured by an initial course of anti-*H. pylori* therapy . Viability of *H. pylori* on gastric mucosal biopsies under various transport conditions, however, has not been studied extensively. Nonetheless, the following may serve as initial guidelines. Sterile saline at room temperature is an adequate medium for up to 6 h,[158] and possibly up to 24h[158] (although refrigeration beyond 6 h is recommended). Saline has the advantage of simplicity and ready availability. Stuart's medium may also be used. Transport to a remote laboratory, however, may require 24±48 h. Transport of a gastric biopsy in saline packed in ice may be adequate provided the saline itself is not frozen. Stuart's medium and glycerol containing media (skimmed milk with glycerol or brucella broth with glycerol) may be more reliable and practical as they may be sent either frozen or refrigerated with dry ice.[159]

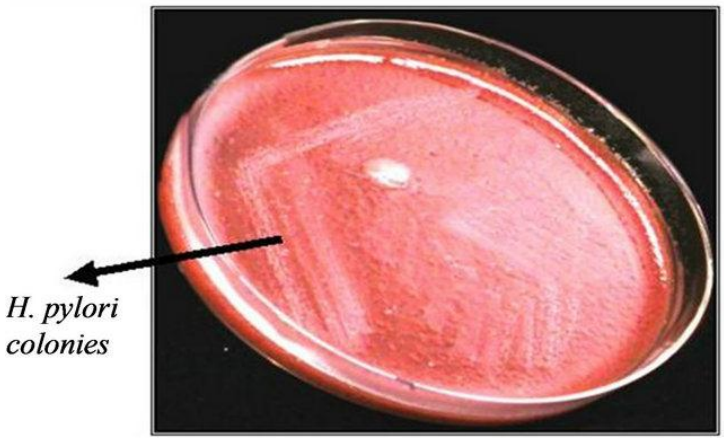

Fig. (16): The *H. pylori* COLONIES on a blood agar

3. Rapid Urease test:

Biopsy Urease tests are quick and easy tests, that indicate only the presence or absence of *H. pylori*, but often have a higher sensitivity than other biopsy tests because the entire biopsy specimen is placed in the media thereby avoiding the additional sampling or processing error associated with histology or culture. They also often allow rapid confirmation of infection (within 30–60 min) at the time of endoscopy although for maximum sensitivity the test should be read 24 h later.[157]

4. PCR:

Molecular methods like polymerase chain reaction (PCR) have the potential to accurately determine both the presence of infection and the genotype of bacteria, and have marked sensitivity and specificity.[160,161] These techniques have been used successfully to detect *H. pylori* DNA in gastric tissues by amplifying genes such as the adhesin genes,[162] the urease gene,[163] and the 16S rRNA gene.[164] The 16S rRNA gene of *H. pylori* is a highly specific target for amplification and has been used previously to help reclassify the organism. The 16S rRNA is one of the specific targets to confirm *H. pylori* infection, and positive amplification of *H. pylori* specific DNA may be considered as a direct evidence of the presence of the pathogen.[165]

B. Non invasive methods:

1. Urea breath test:

H. pylori can be detected noninvasively by the nonradioactive 13C-urea breath test (13C-UBT) which has been used extensively in the development of new drug regimens for *H. pylori*.[166,167] It exploits the abundant Urease activity of *H. pylori*, which rapidly hydrolyses an ingested solution of 13C-urea, to release $13CO^2$, which is then absorbed and excreted as $13CO^2$ in the expired breath. The 13C-UBT can be used to assess several important aspects of infection during the early stages of clinical development of new chemical entities active against *H. pylori*. Nonetheless several groups have demonstrated a relationship between the extent of $13CO^2$ excretion and culture or histology.[168] In addition there is no correlation between the specific Urease activity of isolated strains in vitro with hydrolysis of 13C-urea in vivo (13C UBT), implying that differences in UBT values are due to differences in the numbers of infecting organisms and thus the extent of colonization. Intra-subject quantitative comparisons are possible and semiquantitative assessments made if necessary. [169]

If the 13C-UBT is used to monitor the load of *H. pylori* , suppression is defined as more than 50% fall in the excretion of $13CO^2$. This not only allows rapid and easy assessments of differences between similar agents, which is of particular value for volunteer studies, but also allows more effects on *H. pylori* to be monitored.(170) Thus the effect of lowering intragastric pH on *H. pylori* by omeprazole was first observed with the 13C-UBT that, unlike the antral biopsy, accurately demonstrated suppression, but not clearance of infection.(171)

Clearance of *H. pylori* is defined as a negative 13C-UBT immediately after finishing treatment.(172) Differences in clearance rates will thus reflect differences in eradication rates or rates of recurrence. When assessing clearance with the 13C-UBT it is important to record the time interval between the last dose and the test. Thus even after 1 month of colloidal bismuth or triple therapy some patients, despite suppression of *H. pylori* during treatment, will have a positive breath test less than 12 h after the last dose.(173)

Serial 13C-UBTs at weekly or twice weekly intervals will show the rate of recurrence of *H. pylori* following clearance of infection at the end of treatment. Drugs with the slowest recurrence rates will be the most effective in eradicating *H. pylori* . The 13C-UBT is the best method of following eradication of bacteria in patients.(156,157) Because eradication of *H. pylori* is associated with resolution of histological gastritis and prevention of relapse of DU, the 13C-UBT can be used as the sole method of follow-up. Long-term follow up studies of eradication documented by multiple endoscopic and UBT assessments of *H. pylori* status over a 12-month period showed almost complete concordance, with less than 1% of patients having discordant results.(174)

Fig. (17): The method of Urea breath test

2. Stool antigen test:

Stool antigen testing is a relatively new methodology that uses an enzyme immunoassay to detect the presence of *H. pylori* antigen in stool specimens. A cost effective and reliable means of diagnosing active infection and confirming cure, such testing has a sensitivity and specificity comparable to those of other non invasive tests.(175)

Fig. (18): The stool Antigen test for H. pylori

3. Other serological methods:

During *H. pylori* infection, both local and systemic, humoral and cellular immune responses occur, being ineffective in the elimination of this bacterium. Individuals infected with *H. pylori* produce serum anti-*H. pylori* antibodies IgM, IgA, and IgG classes. Anti *H. pylori* IgM antibodies can be detected in the acute phase of infection. Serum IgA and IgG antibodies indicate chronic infection. Anti *H. pylori* IgA antibodies can be detected in about one third of infected subjects, while almost all produce IgG antibodies. Anti-H pylori IgG antibodies persist during the infection and after successful eradication, the level of these decreases by 50% at 6 month compared with pretreatment level.[176,177]

Serologic tests offer a fast, easy, and relatively inexpensive means of identifying patients who have been infected with the organism. However, this method is not a useful means of confirming eradication of H. pylori; several different samples and changes in titers of specified amounts over time would be needed.[178] In addition, few patients become truly seronegative, even after eradication of the organism.[179]

In low-prevalence populations, serologic tests should be a second-line methodology because of low positive predictive value and a tendency toward false-positive results. Serologic tests may be useful in identifying certain strains of more virulent *H. pylori* by detecting antibodies to virulence factors associated with more severe disease and complicated ulcers, gastric cancer, and lymphoma.[180]

There are several serological tests for determining the cagA status of a patient, either as an ELISA test or as a western blot assay. Determination of cagA status has value in epidemiological trials and in studies of pathogenesis but has limited use in clinical practice.[181,182]

Antimicrobial Resistance

Owing to the difficulties of culturing these bacteria, molecular methods are of great interest in the detection of the organism.

Gerrits et al. found that multiple mutational changes in The *pbp1* A gene led to amoxicillin resistance in *H. pylori*, which renders the development of a molecular test

difficult in contrast to cases of clarithromycin and tetracycline resistance.[183] At the same time, Kim et al. confirmed the association of *pbp1* A gene mutations and amoxicillin resistance using sequence analysis.[184]

12-Treatment

Once *H. pylori* are detected in a person with a peptic ulcer, the normal procedure is to eradicate it and allow the ulcer to heal. The standard first-line therapy is a one week "triple therapy" consisting of proton pump inhibitors such as omeprazole and the antibiotics clarithromycin and amoxicillin.[185] Variations of the triple therapy have been developed over the years, such as using a different proton pump inhibitor, as with pantoprazole or rabeprazole, or replacing amoxicillin with metronidazole for people who are allergic to penicillin.[186]

An increasing number of infected individuals are found to harbor antibiotic-resistant bacteria. This results in initial treatment failure and requires additional rounds of antibiotic therapy or alternative strategies, such as a quadruple therapy, which adds a bismuth colloid, such as bismuth subsalicylate.[187] For the treatment of clarithromycin-resistant strains of *H. pylori*, the use of levofloxacin as part of the therapy has been suggested.[188]

Other trials for eradication

The use of probiotics was tried successfully. In humans Lactobacillus gasseri 21 (LG2 I) and in experimental animal Clostridluin butyricum MIYAIRI 588 were shown to eradicate *H. pylori* infection. This is because of the inhibitory effects of the fatty acid (butyric acid) and the lactic acid on the growth of *H. pylori*[189,190].

Lethal bacterial photosensitization either using phthalocyanine and copper vapour pumped dye laser[191] or using haemotporphyrin derivative as a sensitizer[192], were tried in vitro. Neither of these sensitizers has been further evaluated for eradication of *H. pylori* found on gastric mucosa.

13-Prevention

H. pylori is a major cause of certain diseases of the upper gastrointestinal tract. Rising antibiotic resistance increases the need to search for new therapeutic strategies; this might include prevention in form of vaccination.[193] Extensive vaccine studies in mouse models have shown promising results.[194] Researchers are studying different adjuvants, antigens, and routes of immunization to ascertain the most appropriate system of immune protection; however, most of the research only recently moved from animal to human trials.[195] In popular culture, a number of foods may be useful to prevent colonization with *H. pylori* including: green tea, red wine, broccoli sprouts, garlic, probiotics and flavonoids.[196]

Tovey et al has shown that ulcers are much less common in areas where the staple diets are unrefined grains such as village ground millets and pulses but more common in people eating refined grains as white flour and pollished white rice.[194]

14-Vaccines

Vaccines against *H. pylori* could be used as prophylactic vaccines to prevent the infection or as therapeutic vaccines to cure the infection, to improve the eradication

success of standard regimens or to reduce the bacterial density in the gastric mucosa and the risk for emergence of antibiotic resistant strains. In recent years, many attempts, using various *H. pylori* antigens such as urease, CagA, HP-NAP, HspA or combinations, many adjuvants and different routes of immunization have been made to create vaccines against *H. pylori* infection. Although some attempts are promising, no effective and safe vaccine against *H. pylori* is currently available for humans. New directions for immunization with the use of DNA, living vectors, microspheres etc. are currently under evaluation. The vaccination plan and the groups who should receive vaccination are still to be determined, but the vaccination will be useful, especially in developing countries.[195] An intramuscular vaccine against *H. pylori* infection is undergoing Phase I clinical trials, and has shown an antibody response against the bacterium. Its clinical usefulness requires further study.[196] Study of the *H. pylori* outer membrane is important, both for understanding pathogenicity and for development of vaccines, since the outer membrane is involved in adherence to the host epithelium and stimulation of the host immune response. Vaccines should be able to confer preventive and curative immunity on humans. Oral immunization with a recombinant urease given in the absence of a mucosal adjuvant has been unsuccessful in *H. pylori*-infected volunteers (urease is a cytoplasmic enzyme).[197]

However, the recombinant *H. pylori* urease was given with an Escherichia coli heat- labile toxin, provoking diarrhea in the majority of the volunteers (a side effect which disappeared when the dose was reduced), but also causing an increase in urease-specific IgA-producing cells and a decrease in the density of gastric colonization by *H. pylori*.[197] IgA antibodies are expected to play a prominent role in protection., since *H. pylori* is a non-invasive pathogen at the luminal surface of the gastric mucosa. This hypothesis has been supported by the observation that milk IgA protects infants against *H. pylori* infection.[198]IgA and immunoglobulin GI (IgGl) depend on T-helper type 2 (Th2) cells. According to different experiments, immunization is associated with an elevation of IgG, levels, indicative of a Th2 cellular immune response, which might be a significant mechanism. The field of vaccination is still very controversial, and is being extensively studied.[199,200]

Several major research groups are now working on developing a vaccine for *H. pylori* which offers a radically different approach to the management of infection. Protection against infection has been demonstrated in several animal models of *Helicobacter* infection. In addition therapeutic vaccination to treat established infection (an effect probably mediated by breaking tolerance or up-regulating normal immune responses) has also been demonstrated in animals. However human volunteer studies using enteric vaccines (either therapeutic or protective) have, so far, been disappointing. In addition studies aimed at defining the minimum infectious dose resulted in volunteers developing symptomatic gastritis even though a‘nonpathogenic’ strain was used as the innoculum. More recently classical systemic vaccination using three key *H. pylori* antigens (CagA, VacA and NAP) has been undertaken with some encouraging initial results.[201]