4. Discussion
4.0 Discussion

*Helicobacter pylori* is the causative factor for gastritis, gastric ulcer and MALT lymphoma and gastric cancer in humans. This bacterium finds its niche as the acid coated gastric epithelium and survives there with its special mechanisms. In most of the individuals colonized *H. pylori* keeps a balanced situation of low immune reaction that was not able to eliminate organism but definitely causing tissue damage and which help the bacterium to get sufficient nutrients to survive in the unfavourable environment of the stomach. It is known that over half of the world’s population is infected with *H. pylori*, with the highest rates in developing countries (Rothenbacher D and Brenner H, 2003). Infections occur in early childhood and persist for decades in the absence of targeted antimicrobial therapy. The exact mode of transmission of *H. pylori* is not clearly known till today. New infections thought to occur through human to human contact either in oral-oral or faecal-oral route or both. *H. pylori* is reported to be colonized in few hosts such as humans and some non-human primates and there exists a few reports of isolation of the organism from pet animals (Brown LM et al, 2001; Brown LM et al, 2002; Dore MP et al, 2001; Herbarth O et al, 2001). Although *H. pylori* colonized individuals are high in population, only a few percentage shows clinical symptoms of *H. pylori* associated diseases. Among the *H. pylori* positive individuals 10 to 20% develop ulcer diseases and 1 to 2% having risk of developing gastric cancer or MALT lymphoma (Ernst PB and Gold BD, 2000; Parsonnet J and Issacson PG, 2004). Gastric ulcer patients are characterized by reduced gastric acid secretion, corpus predominant pangastritis, and accelerated progression toward atrophic gastritis and intestinal metaplasia such individuals are reported to be at higher risk for gastric cancer than are duodenal ulcer patients (Hansson LE et al, 1996). 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

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metaplasia occurs in approximately half of the *H. pylori*-colonized population (Kuipers EJ et al, 1995b). 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 risk is not only increased in individuals colonized with cytotoxin associated gene (cagA) positive strains, but also in those with a genetic predisposition to higher IL-1 production in response to colonization (Parsonnet J et al, 1997; El-Omar EM et al, 2000).

*H. pylori* induced ulcer disease, gastric cancer, and lymphoma are complications of chronic inflammation; ulcer disease and gastric cancer in particular occur in those individuals and at those sites with the most severe inflammation. Infection is associated with production of proinflammatory cytokines which lead to chronic or chronic active gastritis. The activity of the gastritis is commonly considered an indicator of severity, and chronic active gastritis is associated with more severe manifestations of disease, such as peptic ulceration and neoplasia (Blaser MJ et al, 1995; Crabtree JE, 1998; Peek RM et al, 1995). The *cag* pathogenicity island (PAI), a 40 kb stretch of DNA-encoding homologues of components of a T4SS, positive strains are associated with severe disease conditions (Censini S et al, 1996). *H. pylori* pathogenesis and virulence factors such as cagPAI and its effector protein cagA (Covacci A et al, 1993), vacuolating cytotoxin (vacA) (Cover TL, 1996), Urease (van Vliet AHM et al, 2001), adhesins such as blood group antigen binding adhesin (BabA), sialic acid binding adhesin (sabA and SabB) (Prinz C et al, 2001), adherence-associated lipoprotein (AlpA and AlpB), *Helicobacter* outer membrane protein Z (HopZ), outer membrane inflammatory protein A (OipA) (Yamaoka Y et al, 2000) and neutrophil activating protein (Nap) (Tonello F et al, 1999; Namavar F et al, 1998), other factors associated with ulcer such as induced upon contact with epithelium gene (IceA) (Figueiredo C et al, 2000) and duodenal ulcer associated protein (DupA) (Lu H et al, 2005), LPS and flagella contributes to the outcome of the gastric pathologies independently or collectively. The cholesteryl-α-
The glucosides of *H. pylori* support the pathogenicity of this organism, because inhibition of the cholesterol glucosyltransferase (*Cgt*) by *O*-glycans of the human gastric mucosa suppresses growth of the bacterium (Kawakubo *et al.*, 2004). Intrinsic α-glucosylation of cholesterol abrogates phagocytosis of *H. pylori* and subsequent T-cell activation (Wunder *et al.*, 2006). Many of these defined *H. pylori* virulence factors, including flagella, the stomach acid neutralizing enzyme urease and the multifunctional VacA, are found in all strains (Monack *et al.*, 2004).

The components of innate immunity such as PRRs play an important role on detecting PAMPs on microbes and orchestrating the adaptive immunity against infection. Toll like receptors constitute a major component of the innate immune system by detecting molecules like lipopolysaccharides (LPS), lipoprotein, lipotheichoic acid, peptidoglycan, lipoarabinomannan (LAM), flagellin, CpG containing DNA, single and double stranded RNA of viruses, HSP60, Porins, Zymosan, Glycolipids are known examples of PAMPs (Janeway CA and Medzhitov R, 2002; Takeda Y *et al.*, 2003). PAMPs recognition by PRRs leads to different signalling events that ultimately exert the expression of many effector molecules of the immune system. The recognition in phagocytic cells leads to the production of reactive oxygen and nitrogen intermediates (ROI & RNI) and increased expression of co-stimulatory molecules which act as second stimuli for T-cells activation. PRRs also signals the production of immune mediators such as chemokines, cytokines, leukotrienes, prostaglandins and anti-microbial peptides, which ultimately determines the recruitment of leukocytes at the site of infection and the production of successful adaptive immunity to eliminate the infection.

Gastric epithelial cells do not express all ten TLR molecules in humans. The invading immune cells are capable of providing the missing receptors for detecting important molecular patterns of many bacteria. *H. pylori* infection attracts more neutrophils and monocytes to the site of infection through induction of IL-8 release and *H. pylori* Nap protein has also been implicated for the neutrophil activating process by
inducing NADPH oxidase (Tonello F et al, 1999; Namavar F et al, 1998; Satin B et al, 2002; Naumann M and Crabtree JE, 2004). The phagocytic cells at the site of infection try to eliminate the microbes by engulfment and subsequent killing by the help of ROIs and RNIs. However, *H. pylori* possess catalase and superoxide dismutase enzymes involved in the ROI scavenging, which might help the organism to evade its elimination by phagocytotic killing (McGee DJ and Mobley HL, 1999). It also produces the arginase enzyme which converts arginine to ornithine and thereby competitively inhibiting the inducible nitric oxide synthase enzyme of the host and control the production of NO. *H. pylori* also induce eukaryotic arginase II expression in macrophages, which might further counteract NO production by these cells (Gobert AP et al, 2002). These mechanisms might limit the availability of L-arginine at the site of infection and thereby control phagocytic killing.

The role of innate immune response to *H. pylori* through PRRs is not completely understood till today. A few studies have reported the involvement of some TLRs in the detection of *H. pylori*. Smith MF et al reported that gastric epithelial cells recognize and respond to H. pylori infection at least in part through TLR-2 and TLR-5 (Smith MF et al, 2004). TLR-4 mediated recognition of bacterial LPS is a key activator of the innate immune response in epithelial cells, while *H. pylori* LPS is a relatively weak inducer. However, *H. pylori* LPS activates NF-κB through TLR-2 rather than TLR-4 (Smith MF et al, 2004; Maeda S et al, 2001; Backhead F et al, 2003). In the present study, the THP1 monocytic leukaemic cell line reported to be expressing all ten TLRs (Zarember KA and Godowski PJ, 2002), THP-1 derived DCs, HEK293 cells having less or no TLR expression and HEK293 cells stably transfected with TLR2, TLR5 and TLR10 have been used to delineate the role of *H. pylori* virulence genes on TLR detection and signalling through in vitro infection assays involving wild type strain and isogenic mutants of cagA, vacA, flaA, cgt and cagPAI.
4.1 Toll like receptors Expression and IRAK-1 phosphorylation during *H pylori* infection in THP-1 cells, THP-1-DCs, HEK-293, HEK293-TLR2, HEK293-TLR5 and HEK293-TLR10 cells

The complete profile of TLR-1 to 10 mRNA expression pattern during *H. pylori* infection is not available. Hence one of the objectives of the present study was to analyze the TLR-1 to 10 mRNA expressions in human monocytic THP1 cell line during *H. pylori* infection. The mRNA expressions of genes give a rough idea of their involvement in cellular activities at a particular event. The higher expression of mRNA of a particular gene attributes to higher turn over or continuous signal transduction of particular pathway that regulate the gene expression. TLRs are involving in the recognition of PAMPs present on microbes or invading organisms and subsequent signalling to activate the pro-inflammatory changes to control infection. Several known ligands from different sources have been reported to bind TLRs and transducing signals. Smith MF *et al* (2004) reported that gastric epithelial cells recognize and respond to *H. pylori* infection at least in part through TLR-2 and TLR-5. *H. pylori* LPS activates NF-kB, through TLR-2 rather than TLR-4 (Smith MF *et al*, 2004; Maeda S *et al*, 2001; Backhead F *et al*, 2003). In contrast to this, Kawahara T *et al* reported that *H. pylori* LPS activated NF-kB in association with the expression of Mitogen oxidase-1 (MOX-1), cyclooxygenase-II (COX II) and TNFα transcripts in gastric pit cells, which express more TLR-4 but no TLR-2 (Kawahara T *et al*, 2003).

The infection of THP-1 cells with *H. pylori* and isogenic mutants of *cagA*, *vacA*, *flaA*, *cgt*, and *cagPAI* at a MOI of 1:50 for 6 and 24h have revealed that virulence factors of *H. pylori* significantly altering the quantitative expression of TLR mRNAs. TLR-1 mRNA expression after 6h was not significantly changed during *H. pylori* infection. However, this level was significantly increased with the infection by wild type, Δ*cgt* and Δ*cagPAI* after 24h of infection (Fig.3). Some investigations have shown that Lipoproteins in which the N-terminal cysteine is triacylated are recognized by TLR-2 in combination with TLR-1. Similarly, diacylated lipoproteins are recognized by TLR-2 in combination
with TLR-6 (Takeda Y et al, 2003; Ozinsky A et al, 2000). CagA, VacA and FlaA proteins of *H. pylori* are required for the increase in cellular mRNA expression of TLR-1 in THP-1 cells. It has been reported that LTA from Group B Streptococcus (GBS) and *S. aureus* have interacted with TLR-2 and TLR-6, but not TLR-1 (Henneke P et al, 2005). TLR-2 expression has been significantly increased by wild type strain but ΔvacA and ΔflaA mutants showed no significant change after 6 and 24 h of infection. However, the mRNA expression level after 6 h of infection was reduced by half after 24 h of infection with *H. pylori* wild type. This may be due to the overall control mechanisms of expression of TLR-2 molecules in THP-1 cells or due to tolerance as reported earlier in the case of TLR-4 and TLR-5 mediated detection of LPS and flagellin, respectively (Mizel SB et al, 2002). In addition, *H. pylori* LPS found to be the ligand for TLR-2 (Smith MF et al, 2004; Maeda S et al, 2001; Backhead F et al, 2003). The above observation has been confirmed by analyzing the TLR-2 mRNA expression in HEK293-TLR2 cells by showing increased expression of TLR-2 by *H. pylori* infection. This experiment demonstrated that ΔvacA and ΔflaA mutants were the least inducer of TLR-2 mRNA expressions. Hence, it can be assumed that TLR-2 ligand (s) from *H. pylori* may need the virulence factor VacA and FlaA for maximum potency and which may be mediated through the changes exerted by VacA on host cells or changes in surface properties of *H. pylori* by FlaA molecules, since *H. pylori* FlaA is a UDP-GlcNAc-inverting 4,6-dehydratase enzyme catalyzes the first step in the biosynthetic pathway of a pseudaminic acid derivative, which is implicated in protein glycosylation (Ishiyama N et al, 2006).

TLR-3 binds known ligand dsRNA from virus and induces IRF-3/7 dependent upregulation of Type-I IFNs (Oganesyan G et al, 2006). However, there are only few reports on bacteria dependent Type-I IFNs production. Gratz N et al (2008) reported that in mouse macrophages, extracellular Gram-positive human pathogen group A streptococcus (GAS; *S. pyogenes*) causes IRF-3 dependent, MyD-88 independent production of type-I IFN and which is also induced by GAS lacking *slo* and *sagA*, the
genes encoding cytolysins that were shown to be required for IFN production in response to other Gram-positive bacteria (Gratz N et al, 2008). THP-1 monocyte infection with *H. pylori* significantly increased the TLR-3 mRNA expression after 6h and continued to increase even after 24h. This shows the important role for TLR-3 in the detection of *H. pylori*. Among the isogenic mutant studied, Δ*flaA* demonstrated least significant change in TLR-3 mRNA expression after 6h and 24h of infection in THP-1 cells. All the other mutants significantly increased the TLR-3 mRNA expression but found to be less potent than wild type strain.

The data regarding the involvement of TLR-4 in *H. pylori* infection are contradictory. *H. pylori* LPS activates NF-κB, through TLR-2 rather than TLR-4 (Smith MF et al, 2004; Maeda S et al, 2001; Backhead F et al, 2003). In contrast to this, Kawahara T et al (2003) reported that *H. pylori* LPS activated NF-κB in association with the expression of Mitogen oxidase-1 (MOX-1), cyclooxygenase-II (COX II) and TNFα transcripts in gastric pit cells, which express TLR-4 without TLR-2 (Kawahara T et al, 2003). Mandell L et al reported that the purified form of *H. pylori* LPS induced cytokine production was mediated through TLR-4, but the response to Helicobacters such as *H. pylori, H. hepaticus, and H. felis* was mediated through TLR-2 (Mandell L et al, 2004).

THP-1 cells have not significantly increased TLR-4 mRNA expression after 6h of infection with *H. pylori*. However, there was a significant increase in TLR-4 mRNA expression after 24h of infection with the wild type and Δ*cgf*. On the contrary, other mutants clearly reduced TLR-4 mRNA expression compared to wild type strain. This indicates that *H. pylori* up-regulate TLR-4 at the late phase of infection, where TLR-2 found to be down-regulated from the initial phase of infection (Fig.2, 3). This would suggest that TLR-4 up-regulation might be occurred due to the LPS released from the bacteria lysis at the late phase of the infection. LPS on the surface of *H. pylori* may have special features which inhibit the interaction with TLR-4, conversely it is able to bind with TLR-2 and induce signal transduction.
Bacterial flagellins are the known ligands for TLR-5. A study using recombinant flaA and ΔflaA mutant of *H. pylori* revealed the less influence of TLR-5 mediated IL-8 secretion in epithelial cells (Gewirtz AT *et al*, 2004). A recent report showed the site responsible for low TLR-5 mediated activity to amino acids 89-96 of the N-terminal D1 domain of *H. pylori* flaA (Andersen-Nissen E *et al*, 2005). THP-1 cells infected with *H. pylori* have up-regulated the TLR-5 mRNA expression significantly. Among the mutants tested, ΔflaA has no significant influence on TLR-5 expression compared to uninfected cells. However, other mutants were less potent than the wild type on inducing TLR-5 mRNA expression. In this study, we found that TLR-5 mRNA expression in THP-1 cells was moderately increased after *H. pylori* infection. This finding was confirmed at the protein level by western blot analysis of immunoprecipitated TLR-5 from THP-1 cells infected with *H. pylori* wild type and ΔflaA mutant. This data strongly suggest the involvement of TLR-5 in *H. pylori* detection and pro-inflammatory changes. In the experiment using HEK293-TLR5 cells infected with *H. pylori* revealed that wild type and ΔcagA mutant up-regulated the TLR-5 mRNA expression significantly, leading to suggest the involvement of TLR-5 in *H. pylori* infection.

TLR-6 binds to ligands such as diacyl lipopeptides from mycoplasma, LTA from Gram positive bacteria and zymosan from fungi (Takeuchi O *et al*, 2001; Schwander R *et al*, 1999; Ozinsky A *et al*, 2000). THP-1 cells infected with *H. pylori* and isogenic mutants significantly up-regulated mRNA expression of TLR6, except in the case of ΔflaA mutant. The wild type strain induced the up-regulation of TLR-6 expression significantly compared to other mutant strains after 6h of infection. However, Δcgt and ΔcagPAI mutants have up-regulated TLR-6 to the level of wild type after 24h of infection. Ozinsky A *et al* (2000) reported that TLR-2 and TLR-6 heterodimer detect the PGNs while TLR-2 homodimer detects bacterial lipopeptide. They also showed that the cytoplasmic domain of TLR-2 can form functional pairs with TLR-6 or TLR-1, and this interaction led to cytokine induction. Ray A and Biswas T (2005) observed that porin of *S. dysenteriae*
type 1 increased the mRNA levels for TLR-2 and TLR-6 and CD80 and also induced cell-surface expression of immunoglobulin IgM, IgG2a, and IgA in peritoneal cavity B-2 cells. Mycoplasmal membrane diacylated lipoproteins initiated proinflammatory responses through TLR-2 and TLR-6 through the activation of NF-κB and also induced apoptotic responses (Into T et al, 2004).

TLR-7 and -8- are involved in the binding of synthetic compounds imidazoquinoline and ssRNA from viruses. TLR-7 also binds synthetic compounds Loxoribine and Bropirimine (Heil F et al, 2003; Heil F et al, 2004; Hemmi H et al, 2002; Jurk M et al, 2002). IFN induction in pDC was triggered by signal transduction pathways through TLR-7 and TLR-9 as well as by recognition of cytosolic virus-specific patterns (Schlender J et al, 2005). No reports are available in the literature showing that bacterial products can bind and induce TLR-7 and -8- mRNA expression. The present study shows that THP-1 cells infected with H. pylori very significantly up-regulated the TLR-7 mRNA expression even after 6h of infection and reached more than 70 fold in case of wild type infected cells compared to uninfected cells. ΔcagA mutant have exhibited an increase in the TLR-7 mRNA expression after 6h when compared to wild type infected cells. However, after 24h of infection, TLR-7 mRNA expression level reached almost double in wild type infected cells when compared ΔcagA mutant infected cells. ΔflaA mutant was the least inducer of TLR-7 mRNA expression. Triantafilou K et al (2005) reported that group B coxsackievirus induced inflammatory response is mediated through TLR-8 and to a lesser extent through TLR-7. TLR-8 and TLR-7 also function as the host sensors for human parechovirus-1, a ssRNA virus (Triantafilou K et al, 2005b). Both type-I IFNs and IRFs are well characterized in viral infections but not in bacterial. S. aureus LTA activated IRF-2 resulted in the up-regulation of IRF-1 and activation of STAT-1 and STAT-3 yielded rapid secretion of IFNα. (Liljeroos M et al, 2008). Interestingly, THP-1 cells infected with H. pylori have up-regulated TLR-8 mRNA expression significantly after 6h of infection and continued even after 24h. ΔflaA mutant
has also significantly up-regulated TLR-8 mRNA expression but weakly after 24h of *H. pylori* infection.

CpG DNA is the known and well studied ligand for TLR-9. LPS has induced cytokine production from DCs in a MyD-88 dependent pathway and also induced functional maturation of MyD-88 (-/-) deficient DCs, including up-regulation of costimulatory molecules and enhancement of APC activity. However, MyD-88 (-/-) deficient DCs could not mature in response to bacterial DNA, the ligand for TLR-9, indicating that MyD-88 is differentially required for signalling of TLR family (Kaisho T et al, 2001). Schmausser B et al (2004) reported that immunocytochemical studies using gastric mucosal biopsies have revealed TLR-5 and TLR-9 expression on the gastric epithelium changed to exclusive basolateral localization without detectable expression at the apical pole in *H. pylori* gastritis. Although the level of mRNA expression was very low, the wild type significantly up-regulated the TLR-9 mRNA expression in THP-1 cells after 6h of infection. Other mutants studied have not significantly changed the mRNA expression after 6h of infection. ∆cagA and ∆cagPAI significantly increased the TLR-9 mRNA expression only after 24h of infection, whereas the wild type infected cells continued to up-regulate TLR-9 expression from 6h onwards. This indicates that the *H. pylori* induced TLR-9 expression moderately increased during *H. pylori* infection and the virulence factors such as VacA, FlaA and CG were found to be essential for up-regulation of TLR-9 expression. *M. tuberculosis* infected TLR9 (-/-) but not TLR2 (-/-) deficient mice displayed defective mycobacteria-induced IL-12p40 and IFNγ responses in vivo, whereas, TLR2/9 (-/-) mice displayed markedly enhanced susceptibility to infection in association with combined defects in proinflammatory cytokine production (Bafica A et al, 2005).

TLR-10 is known as orphan receptor among TLRs. The ligand for TLR-10 is not known till today. No published data is available describing TLR-10 involvement in any infectious and inflammatory process. Normal and neoplastic human B-cells express a
distinct TLR repertoire including TLR-9 and TLR-10 and such expression is increased upon engagement of the antigen receptor complex or ligand with TLR-9 (Bourke E et al, 2003). It has also been reported that TLR-10 is a functional receptor, which can form homodimers and heterodimers with TLR-1 and TLR-2, transducing signals in MyD-88 dependent pathway (Hasan U et al, 2005). THP-1 cells express low levels of TLR-10 mRNA. Infections of THP-1 cells with H. pylori have very significantly increased the TLR-10 mRNA expression after 6 and 24h. All the mutants used in this study have also significantly increased the TLR-10 mRNA expression in THP-1 cells after 24h of infection. As observed in other TLR molecules expression, ΔflaA found to be the least inducer of TLR-10 mRNA expression. The mRNA level expression during H. pylori infection was checked at the protein level by western blot of immunoprecipitated TLR-10 from THP-1 cells infected with H. pylori wild type and ΔflaA mutant. Western blot analysis revealed the high level expression of TLR-10 protein during H. pylori infection. This observation was again confirmed at the mRNA level in HEK293-TLR10 cells infected with H. pylori. In this experiment, H. pylori wild type significantly increased the TLR-10 mRNA expression after 6h of infection. However, among the mutants Δcgt only increased TLR-10 mRNA expression significantly in HEK293-TLR10 cells although not to the level induced by wild type. This attributes that TLR-10 is playing an important role in the detection of H. pylori infection and is the first report on TLR-10 mRNA and protein which can be induced by a bacterium.

THP-1 derived DCs were also used in this study to understand the differential expression of TLRs compared to THP-1 monocytes. LTA and CpG DNA were additive in induction of TNFα, IL-6 and NO in RAW-264 macrophages, peritoneal macrophages and DCs. In contrast, LTA suppressed IL12p40 secretion induced by CpG DNA in RAW-264 cells and peritoneal macrophages but not in DCs. These findings indicated that the consequences of interaction of innate immune cells with microbial pattern depend on the responding cell type and might be differential for certain effector mechanisms (Dalpke
AH et al, 2002). Infection of THP1-DCs with wild type *H. pylori* and ∆*flaA* revealed that TLR-3, TLR-7 and TLR-10 are the three main TLRs very significantly induced in the THP1-DCs during *H. pylori* infection. TLR-1, TLR-2, TLR-5 and TLR-8 mRNA expressions were significantly increased, although the induction was moderate in THP1-DCs during *H. pylori* infection. TLR-6 and TLR-9 mRNA expressions were very weak in THP1-DCs even after 24h of infection with *H. pylori*. TLR-2 expression was moderately increased after 6h of infection with *H. pylori* but found to be decreased after 24h of infection. TLR-6 and TLR-8 were drastically down-regulated in THP-1 derived DCs in comparison with THP-1 cells during *H. pylori* wild type infection. ∆*flaA* mutant was again found to be a weak inducer of TLR mRNA expression. This affirms the earlier findings that TLR expression can be varied in different cell types during the infection with same bacterium.

The TLR family members are capable of recognizing several classes of pathogens and orchestrating appropriate innate and adaptive immune responses. The role of TLRs in innate immunity to *H. pylori* infection was poorly understood. The present study focused to examine the role of all ten known TLRs expression in THP-1 cells and in particular the role of few individual TLRs by stably transfected in HEK293 cells. mRNA expression has revealed that TLR-3, TLR-6, TLR-7, TLR-8 and TLR-10 transcripts were very significantly up-regulated after 6h and 24h of infection in THP-1 cells. TLR-2 and TLR-9 were weakly up-regulated by *H. pylori* in THP-1 cells after 6h of infection. However, TLR-1 and TLR-9 mRNA expressions were moderately up-regulated after 24h of infection. Among the mutants studied, ∆*flaA* and ∆*vacA* found to be the least inducers of TLR mRNA expression in THP-1 cells. Infection of *H. pylori* with HEK293-TLR2, HEK293-TLR5 and HEK293-TLR10 cell lines have also revealed the similar pattern of the aforementioned TLRs and the role of *H. pylori* virulence factors in the establishment of pathogenesis.
IRAK1 phosphorylation is an important event in the downstream signaling after activation of TLRs through ligand binding. Initial phosphorylation of two threonine residues of IRAK1 by IRAK4 induces massive autophosphorylation and dissociation from the MyD88 adapter complex to induce further downstream signaling that allows NFκB activation and pro-inflammatory gene expression (Kollewe C et al, 2004; Akira S and Takeda K, 2004). The IRAK-1 phosphorylation is a critical step in the signalling of TLR proteins. After analysing mRNA expressions of all ten TLRs qualitative changes in the TLR signalling during *H. pylori* infection were also studied. It has been revealed that *H. pylori* virulence and pathogenecity factors such as CagA, VacA, FlaA, Cgt and cagPAI status are essential for IRAK-1 massive phosphorylation in THP-1 cells. IRAK-M level, negative regulator of TLR signalling, was also examined. It was found that IRAK-M levels were not changed significantly in THP-1 cells after infection with *H. pylori*. HEK293-TLR2 cells infected with *H. pylori* and mutants have induced a low level IRAK-1 phosphorylation. In HEK293-TLR5 cells infection, Δcgt and ΔcagPAI mutants did not induce IRAK-1 phosphorylation at all, however, wild type, ΔcagA, ΔvacA and even ΔflaA induced low level of IRAK-1 phosphorylation. Interestingly, HEK293-TLR10 cells had induced massive IRAK-1 phosphorylation during infection with *H. pylori* wild type strain, whereas very low level of phosphorylation was noted with ΔflaA and ΔvacA mutants. HEK293 cells having no or less TLR expression did not activate IRAK-1 phosphorylation during *H. pylori* infection. This implies that *H. pylori* induce IRAK-1 massive phosphorylation through TLR molecules in a host cell type dependent mechanism.

4.2 Nod like receptors Expression and Functional Activation of Inflammasome for IL-1β maturation and secretion during *H pylori* infection with THP-1 monocytes

Nod-like Receptors are the cytoplasmic pattern recognition receptors for detecting microbial molecular patterns and transducing signals to mount a pro-inflammatory reaction. NLR family of proteins are recognized with a modular organization of a C-terminal leucine rich repeats (LRR), central Nucleotide binding domain (NACHT) and an
N-terminal CARD domain, pyrin domain or Bir domain for protein-protein interaction (Tschopp J et al., 2003). These proteins are able to form the high molecular weight structures called ‘inflammasome’ and recruit caspase-1 that leads to proteolytic activation of pro IL-1β and pro IL-18 (Martinon F and Tschopp J, 2004; Martinon F et al., 2002). The mRNA expressions of NLRs molecules were not up-regulated more than 2 fold in THP-1 cells during H. pylori infection, except IPAF. The mRNA expression of IPAF was significantly increased by Δcgt, ΔcagA and wild type after 6h of infection. However, wild type and isogenic mutants infected cells have significantly increased the IPAF expression after 24h. The ΔvacA mutant was the least inducer of IPAF in H. pylori infected THP-1 cells. This reveals that IPAF might be playing an important role in the intracellular detection of H. pylori or its derived molecules in THP-1 cells. It has been reported that NLRs such as NALP-1, NALP-3 and IPAF forms ASC-containing inflammasomes, which activates Pro-caspase-1 to process Pro-IL-1β to mature form (Martinon F and Tschopp J, 2004; Martinon F et al., 2002). ASC or PYCARD is a protein that is essential for LPS-induced activation of caspase-1, because ASC knockout mice fail to process procaspase-1 or produce IL-1β and IL-18 following LPS and ATP stimulation, which is consistent with the results obtained in Caspase-1 null mice (Mariathasan S et al., 2004; Li P et al., 1995; Kuida K et al., 1995). However, ASC mRNA expression was very significantly down-regulated in THP-1 cells infected with H. pylori. This might have serious implications on the inflammasome Pro-IL-1β and Pro-IL-18 processing and subsequent secretion. ASC was found to be essential for the secretion of IL-1β/IL-18, but dispensable for IL-6, TNFα and IFNγ production, in macrophages infected with L. monocytogenes. Activation of caspase-1 was abolished in ASC deficient macrophages, whereas activation of NF-κB and p38 was unaffected. In contrast, secretion of IL-1β, IL-6, and TNFα was reduced in TLR2-deficient macrophages infected with L. monocytogenes (Oezoeren N et al., 2006). The secretory forms of these cytokines, IL-1β and IL-18, are synthesized as biologically inactive precursor molecules.
inside cells and are cleaved by the enzyme caspase-1 to the biologically active mature forms that are released from cells. IL-1β is synthesized by multiple cells including monocytes, macrophages, neutrophils, hepatocytes, and tissue macrophages throughout the body (Arend WP et al., 2008). Western blot analysis had revealed that mature IL-1β were not significantly changed in THP-1 cells during infection with *H. pylori*. However, western blot analysis had shown that ∆cagPAI, ∆cagA and ∆cgt mutants were able to atleast activate Pro-caspase-1 cleavage to active caspase-1, this step is necessary for Pro-IL-1β cleavage to form mature IL-1β. The above data would suggest that inflammasome components expression and functional process have been severely deranged in THP-1 cells during infection with *H. pylori*.

IL-1β lack signal peptides, or leader sequences, and are not released from cells by the usual mechanism of vesicular transport form the Golgi apparatus. The secretion of IL-1β from THP-1 cells infected with *H. pylori* found to be very less amount and ∆cagA mutant infected cells have significantly increased the secretion when compared wild type and other mutant infected cells. This is showing that CagA molecule is some way inhibiting the secretion of IL-1β. It has been reported that SRC family tyrosine kinase inhibited the neurotransmitter release from neuronal cells (Ohnishi H et al., 2001). CagA is recognized as a substrate by SRC and ABL family kinases that phosphorylate it on tyrosine residues in unique C-terminal region EPIYA motifs (Selbach M et al., 2002; Stein M et al., 2002; Poppe et al., 2007; Tammer et al., 2007). The role of SRC in IL-1β secretion was examined by specifically inhibiting the SRC activity using the inhibitor PP2. Additional inhibitors such as pan-caspase inhibitor Z-VAD-FMK and EGFR kinase inhibitor AG1478 were also used to check whether these enzymes are involved in the processing and secretion of IL-1β. It was observed that PP2 treated THP-1 cells had significantly increased the IL-1β secretion even after 8h of infection and continued up to 32h. This would suggest that CagA protein and SRC kinase are involved in the processing and secretion stage of IL-1β. The earlier reported role of IL-1β on inhibition
gastric acid secretion and pangastritis formation may be due to the release of Pro-IL-1β from dead cells and cleaved by some non-specific proteases to mature form.

4.3 Pro-inflammatory cytokine and chemokine expression and secretion in relation to TLR Expression and Signalling during *H. pylori* infection with THP-1 monocytes

Immune mediators such as IL-1β, IL-8 and TNFα proteins and gene polymorphisms have been implicated in the *H. pylori* associated pathologies (El-Omar EM *et al*, 2000; Rad R *et al*, 2003; El-Omar EM *et al*, 2003; Gyulai Z *et al*, 2004). The involvements of innate immune receptors such as TLRs role in cytokine and chemokine gene expression have not been studied extensively. In this study, attempts were made to analyze the pro-inflammatory cytokines such as IL-1β, IL-6, IL-8 IL-18 and TNFα gene expression and secretion in THP-1 cells in relation to TLR expression and signalling during *H. pylori* infection. The particular role of few TLRs on inducing the above mentioned cytokine and chemokine genes have also been analyzed using HEK293-TLR2, HEK-TLR5 and HEK-TLR10 cell lines infected with *H. pylori*.

Three decades ago, TNFα and TNFβ or lymphotoxins (LT) were identified as products of lymphocytes and macrophages that caused the lysis of cells, especially tumor cells (Granger GA *et al*, 1969; Carswell EA *et al*, 1975). TNF secretion can be induced by conserved structural elements common to microbial pathogens, including cell wall moieties such as PGN, LPS, and CpG DNA motifs on binding with TLRs (Aderem A and Ulevitch RJ, 2000). TLRs transcriptionally induce proinflammatory cytokines, including TNF, through the convergence of NF-kB and NF-AT activating pathways, and enhance translational efficiency by a mechanism targeting consensus 39-untranslated AU-rich elements (ARE) in mRNA (Dumitru CD et al., 2000). The ARE is common to many cytokine mRNAs and is bound by tristetraprolin (TTP), a zinc finger–containing protein that accelerates the turnover of ARE-containing mRNAs (Carballo E et al,1998). TTP is induced by TNF as a negative feedback loop that limits TNF activity. Therapeutics to inhibit TNF have been developed which control previously recalcitrant inflammatory
conditions such as rheumatoid arthritis and inflammatory bowel disease (Maini RN and Taylor PC, 2000; Papadakis KA and Targan SR, 2000). THP-1 cells infected with *H. pylori* and isogenic mutants had significantly increased the mRNA expression of TNFα after 6h of infection. However, the level of mRNA expression of TNFα has been significantly decreased in ∆vacA and ∆flaA mutants after 24h of infection when compared to wild type infected cells. TNFα secretion from THP-1 cells after 24h of infection was also analyzed. The data showed that ∆vacA and ∆flaA mutants significantly reduced the secretion of TNFα from THP-1 cells after 24h of infection when compared to wild type infected cells which correlated with mRNA expressions after 24h. In addition, ∆cagPAI mutant also reduced TNFα secretion from THP-1 cells when compared to wild type infected cells. However, secretion of TNFα secretion from THP-1 cells infected with ∆vacA, ∆flaA, and ∆cagPAI significantly increased in comparison with uninfected cells. This suggests that VacA and FlaA molecules, which have previously been shown to induce significantly less TLR expression and signalling, may be partially responsible for the induction of TNFα secretion from THP-1 cells during infection with *H. pylori*. TLR expression and signalling might be playing an important role along with other signal transduction pathways in TNFα gene expression and secretion during *H. pylori* infection.

It has been reported that activation of all four MAP kinase pathways showed a dramatic synergistic effect on TNF reporter gene expression. The MAP kinases appear to target unknown cis-element(s) in the TNF promoter and ARE in the 3′-UTR (Zhu W et al, 1999).

The IL-1β and IL-18 are activated by inflammasome and have similarity in receptor structure and signal transduction. The receptors for IL-1 are IL-1 receptor-I (IL-1R-I), IL-1R-II and the IL-1R accessory protein (IL-1RAcP). IL-18 has IL-18 receptor and the IL-18R accessory protein (IL-18RAcP). IL-1β is synthesized as 31 kDa biologically inactive precursor molecules inside cells and are cleaved by the enzyme caspase-1 to the biologically active 17 kDa mature form that are released from cells. It is synthesized by multiple cells including monocytes, macrophages, neutrophils, hepatocytes, and
tissue macrophages throughout the body. IL-1β lack signal peptides, or leader sequences, and are not released from cells by the usual mechanism of vesicular transport form the Golgi apparatus (Arend WP et al, 2008). IL-18 is produced by monocytes/macrophages in the presence of different microbial components and plays a major role in the innate immune responses to pathogens. IL-18 is particularly important for the clearance of intracellular pathogens and viruses (Gracie JA et al, 2003). The IL-1β mRNA expressions in THP-1 cells were significantly increased after 6h of *H. pylori* infection. In addition, ∆cagA and ∆cgt mutants infected cells had very significantly increased the IL-1β mRNA expression when compared to wild type infected cells. This may be due to direct involvement of CagA and Cholesterol glucosides on transcriptional regulation of IL-1β during the early phase of infection. However, the wild type strain exhibited more mRNA expression of IL-1β after 24h of infection with THP-1 cells. This type of increase in IPAF mRNA expression by ∆cagA and ∆cgt mutants has been observed in the previous experiments describing the NLR expression. This suggests that CagA and cholesterol glucoside of *H. pylori* may be involved at the transcriptional regulation of host genes. Although *H. pylori* induced IL-1β mRNA expression significantly, the production, processing and secretion of IL-1β at the protein level have been significantly deranged in *H. pylori* infection. In addition, CagA mutant and inhibition of SRC through PP2 treatment and subsequent infection resulted in the increase of IL-1β secretion significantly.

IL-18 was originally identified as an IFNγ inducing factor (IGIF), which circulated during endotoxemia in mice primed with *Propionibacterium acnes* (67). Like IL-1β, IL-18 is synthesized as a 23 kDa biologically inactive precursor peptide, which is subsequently cleaved by caspase-1 (68). Pro-IL-18 is expressed in macrophages, DCs, Kupffer cells, keratinocytes, chondrocytes, synovial fibroblasts, and osteoblasts (Arend WP, 2008). THP-1 monocyte is also expressing IL-18 mRNA and infection with *H. pylori* has not significantly increased the mRNA expression after 6h of infection. However, the IL-18
mRNA expression was significantly down-regulated after 24h of infection of \textit{H. pylori} with THP-1 cells. IL-18 secretion as analysed by ELISA (data not shown) after 24h infection was negligible since no detectable any amount was present in the supernatant collected from THP-1 cells infected with \textit{H. pylori} and isogenic mutants. Hence it can be assumed from the above data, IL-18 expression and secretion are not playing important role in THP-1 cells during \textit{H. pylori} infection.

IL-6 is traditionally considered an activator of acute phase responses and a lymphocyte stimulatory factor (Kishimoto T \textit{et al}, 1995). IL-6 belongs to a family of cytokines that promote cellular responses through a receptor complex consisting of at least one subunit of the signal-transducing glycoprotein gp130 (Heinrich PC \textit{et al}, 2003). Many of the biological activities assigned to IL-6 are mediated through naturally occurring soluble IL-6 receptors (IL-6R). This soluble receptor forms an agonistic complex with IL-6 that binds gp130 to trigger cellular responses. Regulation of this activity is termed “IL-6 trans-signaling” (Jones SA and Rose-John S, 2002; Jones SA \textit{et al}, 2005). In contrast to the ubiquitous expression of gp130, the cognate IL-6R exhibits a highly defined pattern of expression and is largely confined to hepatocytes and leukocytes (Jones SA \textit{et al}, 2001). The mRNA expression of IL-6 in THP-1 cells during \textit{H. pylori} infection was studied and it was found that $\Delta$cagA and $\Delta$cgt infected cells significantly increased the expression after 6 of infection. However, after 24h of infection not only the mutants but also the wild type increased the mRNA expression of IL-6 and the induction by $\Delta$cagA and $\Delta$cgt were more than wild type. $\Delta$vacA, $\Delta$flaA and $\Delta$cagPAI infected cells were the least inducers of IL-6 mRNA expression in THP-1 cells among the strains tested. As seen in the case of IL-1$\beta$ and IPAF, CagA and Cholesterol glucosides are directly involving in the regulation of mRNA expression and that continued even in the late phase of infection.

Interleukin-8 (IL-8) is a potent neutrophil-activating chemokine, central to the immunopathogenesis of \textit{H. pylori} induced tissue injury (Crabtree J and Lindley I, 1994)
and high tissue expression is dependent on the presence of a complete cagPAI (Nilsson et al., 2003). In gastric cell lines, co-culture with *H. pylori* induces IL-8 via activation of NF-κB a transcriptional regulator (Sharma et al., 1998; Nozawa et al., 2002). In the present study, the IL-8 secretion from THP-1 cells after 24h of infection with *H. pylori* was analyzed. *H. pylori* wild type strain induced very high level of IL-8 secretion from THP-1 monocytes. However, ∆flaA and ∆cagPAI mutants significantly reduced IL-8 secretion to 74% and 40%, respectively, in comparison with wild type. It can be concluded that reduced TLR expression and signalling by ∆flaA and ∆cagPAI mutants may be one of the reasons for the reduced IL-8 expression and secretion in THP-1 cells.

**4.4 Pro-inflammatory cytokine and chemokine expression and secretion in relation to TLR Expression and Signalling during *H. pylori* infection with HEK293, HEK293-TLR2, HEK293-TLR5 and HEK293-TLR10 cells**

HEK293 cells are having less or no TLR expression and therefore an ideal cell line to study the role of individual TLRs during a particular infection or inflammatory process. We have used HEK293 cells and HEK293 cells stably transfected with TLR-2, TLR-5 and TLR-10. In this study, we analyzed the mRNA level expression of TNFα, IL-1β, IL-18 and IL-6 after 6h infection of *H. pylori* with HEK293, HEK293-TLR2, HEK293-TLR5 and HEK293-TLR10 cells. HEK293 cells infected with *H. pylori* and isogenic mutants up-regulated IL-8 secretion and TNFα mRNA expression and secretion significantly when compared to uninfected cells. However, the mRNA expression of IL-1β, IL-18 and IL-6 were not increased during infection with *H. pylori* and isogenic mutants. The role of TLR-2, TLR-5 and TLR-10 in pro-inflammatory gene expression and secretion was also studied by infecting stably transfected TLRs in HEK293 cells.

HEK293-TLR2 cells substantially enhanced the mRNA expression and secretion of TNFα in comparison with HEK293 cells infected with *H. pylori* and isogenic mutants. IL-8 secretion from HEK293-TLR2 cells during *H. pylori* infection was reached more than 20 fold of the secretion exhibited by HEK293 cells. The TNFα mRNA expression was highly induced in HEK293-TLR2 cells when compared to HEK293 cells during infection.
with *H. pylori* and isogenic mutants. HEK293-TLR2 cells secreted 3.5 to 5 times TNFα than by HEK293 cells. This enhancement of TNFα expression and secretion in HEK293-TLR2 cells may be due to the increased signal transduction through TLR-2 activation. IL-1β mRNA expression was increased in HEK293-TLR2 cells in comparison with HEK293 cells during infection with *H. pylori* and isogenic mutants. However, IL-6 and IL-18 mRNA expression increased only below two folds in both cell lines and no further enhancement was noted during *H. pylori* infection. This supports the earlier findings that TLR-2 is involved in *H. pylori* infection mediated pro-inflammatory changes. In the present study, IL-1β, IL-8 and TNFα expressions were enhanced in HEK293-TLR2 cells in comparison with HEK293 cells during *H. pylori* infection. ∆cagA and ∆cagPAI mutants infected HEK293-TLR2 cells have exhibited a significant level of reduction in IL-8 secretion when compared to wild type strain. ∆cagA, ∆vacA, ∆flaA and ∆cgt infected HEK293-TLR2 cells have shown significant reduction in TNFα mRNA expression and secretion when compared to the wild type strain. However, TNFα mRNA expression change was not significantly in ∆cagA infected cells (Fig.23,24). IL-1β mRNA expression has significantly reduced in HEK293-TLR2 cells infected with ∆vacA, ∆flaA and ∆cgt mutants, on the contrary, ∆cagA infected cells significantly increased the expression when compared to wild type strain (Fig 22, 23, 24).

HEK293-TLR5 cells infected with *H. pylori* and isogenic mutants enhanced IL-8 secretion almost seven folds when compared to infected HEK293 cells. TNFα mRNA expression increased in HEK293-TLR5 cells in comparison with HEK293 cells during infection with *H. pylori* and isogenic mutants. However, this enhancement was not as high as exhibited by HEK293-TLR2 and HEK293-TLR10 cell lines infected with *H. pylori* and isogenic mutants. TNFα secretion from HEK293-TLR5 was almost similar to the HEK293 cells secretion. This implies that TLR-5 signalling during *H. pylori* infection is not playing much role on inducing TNFα expression. IL-1β, IL-6 and IL-18 mRNA expressions were not increased in HEK293-TLR5 and HEK293 cells during infection with
*H. pylori*. ∆*vacA*, ∆*flaA* and ∆*cgt* mutants infected cells have significantly reduced the IL-8 secretion from HEK293-TLR5 cells when compared to the wild type strain (Fig.25, 26, 27).

The present study revealed the fact that TLR-10 is highly expressed during *H. pylori* infection. The involvement of TLR-10 on cytokine gene expression and secretion has not been studied earlier. HEK293-TLR10 cells infected with *H. pylori* and isogenic mutants have significantly increased the IL-8 secretion, which was observed to be more than 5-7 folds in comparison with HEK293 cells, suggesting that TLR-10 has increased the IL-8 expression and secretion through its signalling. TNFα mRNA expression has significantly increased in HEK293-TLR10 cells infected with *H. pylori* and isogenic mutants. However, TNFα secretion from HEK293-TLR10 in comparison with HEK293 cells was significantly increased in wild type, ∆*cagA* and ∆*cagPAI* infected cells only. IL-1β, IL-6 and IL-18 mRNA expressions in HEK293-TLR10 cells were increased not more than two fold in HEK293-TLR10 cells in comparison with HEK293 cells during *H. pylori* and isogenic mutants infection. This indicates that TLR-10 is a functional receptor and enhancing the IL-8 and TNFα secretion significantly, but not IL-1β, IL-6 and IL-18 mRNA expression, during infection with *H. pylori* and may be playing an important role in the pro-inflammatory changes during this peculiar bacterial infection.

### 4.5 IL-1 Receptor Antagonist polymorphism and genotyping of CagA, VacA, IceA1 and A2 among a group of patients colonized with *H. pylori*

The host genetic background in relation to colonization of *H pylori* was analyzed in the present study. Gastric mucosal biopsy samples collected from patients who have tested positive for rapid urease test under routine endoscopic examination were included as the subjects. The presence of *H pylori* infection was further confirmed by PCR amplification for *GlmM* gene. 42 *H pylori* positive samples diagnosed by above method were further analyzed for genotyping of *VacA* S, *CagA* and *IceA1* and *IceA2*. *VacA* S region was very important in determining the vacoulationg action of *VacA* protein of *H
*pylori*. *VacA* S1 is found to be more toxic than *VacA* S2, which is having an additional 27 bp nucleotide sequence probably affecting the hydrophobic characteristic of mature protein and its secretion. This showed that 74% of patients are infected with *VacA* S1 genotype and associated with 3 peptic ulcer diseases and 29 NUDs, in which one sample is infected with both *VacA* S1 and S2. But there is no significant change was observed in this patient to discuss the importance of this co-existence as reported in few earlier studies (Rahman M *et al.*, 2003). *CagA* gene is a marker of cag pathogenicity island found in certain *H pylori* strains and a crucial virulence factor associated with peptic ulcer diseases and gastric cancer in various studies (Rugge M *et al.*, 1999; Miehlke S *et al.*, 2000, Van Doorn LJ *et al.*, 1998). *CagA* genotype was also analyzed among this group of patients infected with *H pylori*. *VacA* S1/*CagA* genotype is more virulent due to the synergistic effect of these toxic proteins on the host system. Among the patients 45% found to be infected with *CagA* genotype, of which 79% associated with *VacA* S1 genotype and thereby increasing the severity of toxic effects. The only patient having antral ulcer is associated with more virulent *VacA* S1/*CagA* genotype. This genotype is present in 13 other NUDs showing these patients are at more risk for developing ulcers and gastric cancer in later stages if the infection persists.

Host genetic background is also implicated as a putative factor on determining the severity of *H. pylori* associated diseases. Interleukin-1 Receptor Antagonist (*IL-1 RN*) gene is reported to be associated with inflammatory conditions due to its polymorphic region within the second intron containing 2–6 tandem repeats of an 86 bp sequence. In which, the *IL-RN*\(^*2/*/2\) genotype has been associated with proinflammatory responses more severe and more prolonged than those of other *IL-RN* genotypes (Hurme M and Helminen M, 1998). This analysis of *IL-RN* gene polymorphism using specific primers differentiating the alleles among patients was performed. The pro-inflammatory *IL-RN*\(^*2/*/2\) allele was present in 43% of patients studied, while the intermediary heterozygous allele *IL-RN* \(^*1/*/2\) in 36% of patients. *IL-RN*\(^*1/*/1\) allele was present in the
remaining 21% of patients. The high risk VacA S1/CagA/IL-1RA*2-allele were present in 14% of patients studied and VacA S1/CagA/IL-1RN*1/*1 allele in 7% patients, while VacA S1/CagA/IL-1RN*1/*2 allele in 16% of patients. The host pro-inflammatory factors along with bacterial virulence factors may increase the risk for pathological changes leading to gastric cancer among this group of patients. The highest prevalence of gastric abnormalities were reported in patients with both host and bacterial high-risk genotype such as VacA S1/CagA/IL-1RN*2/*2/IL-1B-511T (Rad R et al., 2003).

IceA was identified following transcriptional up regulation on contact with gastric epithelial cells (Peek RM et al., 1998). IceA exists as two distinct genotypes, iceA1 and iceA2, and only iceA1 mRNA is induced following adherence in vitro. H pylori iceA1 demonstrates strong homology to a restriction endonuclease nlaIIIR in Neisseria lactamica (Xu Q et al., 2002), and in vivo carriage of H pylori iceA1 strains has been reported to be associated with peptic ulceration and enhanced acute neutrophilic infiltration. It has been reported that IceA1 genotype is the predominant in the East Asia, while iceA2 genotype is predominant in the USA and Columbia (Yamaoka Y et al., 1999). The present study examined the IceA1 and IceA2 genotypes along with the high-risk host and bacterial factors. Although the protein product of IceA1 and IceA2 has no significant homology to known proteins and its structure reveals patterns of repeated protein cassettes. IceA1 strains have been reported to be associated with peptic ulceration and enhanced acute neutrophilic infiltration, while iceA2 strains are more prevalent among patients with asymptomatic gastritis and non-ulcer dyspepsia. Further analysis revealed that 81% and 19% infected with IceA1 and IceA2 genotype, respectively, and among these 31% patients infected with both strains. IceA1 and IceA2 strains were present in all four peptic ulcer patient samples included in the study. Kidd et al. reported that the vacA S1b, m1 and iceA1 were closely linked to gastric cancer and 40% of patients in their study had infected with iceA1 and iceA2 strains, although higher prevalence of gastric cancer patients infected with iceA1/VacA S1genotype observed in
South African population. Further studies are needed to establish the synergistic effect of VacS1/CagA+/IL-1RA*2/IceA1/IceA2 combination on establishing gastric pathologies in *H pylori* infected patients.