IL-10 polymorphisms in inflammatory and infectious respiratory diseases with seropositive H. Pylori

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ABSTRACT

Background: The critical role of interleukin 10 (IL-10) in susceptibility and resistance to infection is well documented in animal models. However, the single nucleotide polymorphisms in the promoter region of the IL-10 with that of Helicobacter *pylori* infection and inflammatory respiratory diseases warrant an in-depth study and analysis.

Aim: The study therefore aims to address the possible association of IL-10 polymorphism and respiratory disease susceptibility to Tuberculosis [TB], Asthma and Chronic Pulmonary Obstructive Disease (COPD).

Methods: The SNPs -1082 (G/A),-819 (C/T) and -592 (C/A) of IL-10 upstream transcription sites were evaluated by PCR for possible association in a case controlled study in respiratory diseases with serologically positive H. pylori infection.

Result: 1082 (G/A) polymorphism, an ETS transcription binding site of IL-10 demonstrated significant association in Tuberculosis patients with positive H. *pylori* infection.819(C/T) polymorphism did not show any association in all forms of inflammatory lung diseases. There is a heterogeneous polymorphism with 592 (C/A) with the degree of chronic state of the inflammation.

Conclusion: It is the polarization of the immune responses that may be evident in the persistence of the infectious state due to H. pylori.

Key words: helicobacter pylori, interleukin 10 [IL 10], single nucleotide polymorphism (SNP), immune response

INTRODUCTION

There are immunoregulatory mechanisms that are required to control immune response and protect the host from infection.[1,2] Exacerbated immune response due to infection is an effect of imbalance immune repertoire which is not protected. Interleukin 10 (IL-10) is a critical immunoregulatory cytokine with pleitropic effects. It is predominantly anti-inflammatory and has been implicated in a variety of immune and inflammatory diseases.[3] Its role in respiratory diseases is well documented but the association with Helicobacter *pylori* infection has not been corroborated. IL-10 has a dichotomous role of tumor proliferation and suppression.[4,5] Genotypic variations in the human IL-10 promoter may account for individual variation in IL-10 production and, in turn, susceptibility to a particular disease. Single nucleotide polymorphisms (SNPs) of IL-10 transcription site are of increasing interest in associating genetic variations in susceptibility.[6]

The SNPs -1082 (G/A), -819 (C/T), -592 (C/A) are proximal to the IL-10 promoter, upstream of the transcription start site with distinct function i.e., ETS transcription binding site, estrogen receptor element and negative regulatory site respectively.[7,8] An association between tuberculosis susceptibility and polymorphism at position -1082(G/A) in the IL-10 as reported by various groups is controversial and no data is available from tropical countries. It has also been observed that -592 (C/A) of the IL-10 gene are at high risk of HIV-1 infection progressing rapidly to AIDS.[9] Inconsistent results, lack of case controlled studies in a large cohort, discrepancies due to

gene-environment and gene-gene interaction have been major limitations till date to establish strong and specific associations with polymorphism for Helicobacter *pylori* infection.[10] Herein, we report for the first time, the IL-10 polymorphism association in respiratory diseases with sero-positive Helicobacter *pylori* infection.

MATERIALS AND METHODS

Patient selection:

The present study on *H. pylori* was carried out on 106 clinically diagnosed patients of lung inflammatory disorders namely pulmonary tuberculosis and obstructive airway diseases infection, attending as out patients at Chest clinic, Hyderabad, during the period 2006 – 2009. The diagnosis was carried out by Chest X-ray, sputum examination, pulmonary function tests and Spirometry. Institutional Ethical Committee (IEC) approval was taken prior to study. A written informed consent was obtained by all participants of the study.

The prospective study included 2 groups i.e. 42 healthy controls and study cohort of 106 patients including freshly diagnosed, untreated TB (N=58), Asthma (N=21) and COPD (N=27). Blood specimens were obtained from study cohort [106 participants] as well as healthy controls [42 participants]. All the samples were processed for H. Pylori by ELISA to determine the extent of seropositivity. All the sero positive cases were further screened for H. Pylori antigens by immunoblot and also the SNP analysis for the three SNPS of IL-10 (-592,-1082,-819).

Helicobacter pylori sero-positivity by ELISA

Sera from both the groups were tested by H. Pylori ELISA as per manufacturer's instructions. The microtiter wells pre-coated with specific H. Pylori antigens were incubated with 100µl of controls, calibrators and diluted sera, for 30min at 23°C. Antibody recognizing the particular antigen bind during the first incubation followed by the anti-human IgG (gamma chain specific) conjugate reaction. Subsequently the immune complex formation was observed by the chromogen substrate reaction. The O.D. values observed at 450nm demonstrate the load of H. Pylori infection in the individual patient. The semi quantitative method was deemed positive when the ratio is above 1.0 on the calibrator 2 value whereas test ratio >5.0 was considered as strong positive.

Immunoblot

Immunoblot assay was performed in strong positive test ratio (>5.0) cases. These strong seropositive cases were assayed to detect specific antigens prevalent with lung inflammatory diseases. Anti-H. Pylori IgG antibodies in the serum were detected with commercially available western blot strips (EUROIMMUN Mediznische Labordiagnostika, Lubeck, Germany) as per manufacturer's recommendations. The blot strip consisted of antigen extracts with the following molecular weights of the corresponding bands to these proteins which were 120 kDa (CagA); 95 kDa (VacA); 67 kDa (flagellar sheath protein, nonspecific); 66 kDa (UreB); 57 kDa (heat-shock protein homolog); 33 kDa, 30 kDa, 29 kDa (UreA); 26 kDa, 19 kDa and 17 kDa. Specific IgG antibodies bound to the corresponding antigenic site were incubated with enzyme labeled anti-human IgG (enzyme conjugate) diluted 1:10 for 30 min at 23°C. After repeated washing, the bound antibodies were stained with a substrate solution (Nitrobluetetrazolium chloride/5-Bromo- 4-chloro -3indolyl phosphate (NBT/BCIP) for 15 min at 23°C in dark condition. The reaction was observed as an intense dark band at the line of the corresponding antigen considered as positive. The control bands provide a consistent semi quantitative standard against which the reactivity to specific antigens can be measured.

Generating IL-10 Promoter PCR Products

The polymorphism of the interleukin-10 (IL-10) with H. Pylori infection was examined by using the

polymerase chain reaction (PCR) method with genomic DNA and allele-specific primers. Genomic DNA was extracted by using QIAamp DNA Mini Kit (Qiagen). The extracted DNA was quantified by spectrophotometer and stored at -20°C. Three biologically important SNPs -1082 (G/A),-819 (C/T) and -592 (C/A) located in the promoter region of the IL-10 gene were studied in a normal control population and in patients. Primers used to generate DNA products to study the susceptibility of H. Pylori to the SNPs of IL-10 promoter site included were:

SNP-1082(G/A) (192bp) :

(F) 5`CAAGACAACACTACTAAGGC 3`,

(R)5'AATGGCCTTAGAGTTTCTTTTAG 3`

SNP819(C/T)(204bp):

(F)5'TCATTCTATGTGCTGGAGATG3',

(R)5`GAAGTGGGTAAGGTAGTCTG3',

SNP–592(C/A),(319bp):

(F)5`GACTACTCTTACCCACTTCC3',

(R)5`GGATTGAGAAATAATTGGGTCC3'.

Polymerase chain reaction (PCR) reactions were set-up using thermal cycler (Applied Biosystems) with cycler conditions: 94°C for 10 min, 35 cycles of 94°C for 15 sec, 60°C for 15 sec, 72°C for 1 min, and a final extension at 72°C for 7 min. Amplified DNA products containing the specific IL-10 promoter SNPs were electrophoresed through ethidium bromide containing 2% agarose gels, and visualized by UV transilluminator to determine each PCR product against their responding molecular weight.

RESULTS

Immunological characterization of H. Pylori infection:

The sera of the controls and the patients were tested for the presence or absence of H. Pylori infection. The 75% of control groups reacted with the antibodies of H. Pylori on ELISA micro titer plates indicating the infection as a normal microbial flora of the intestine. The subject cohort including the three groups of patients showed positive reaction by ELISA which confirms H. Pylori infection. Results are shown in Table 1.

Table 1: Seropositivity for *H. pylori* infection

Gender	TB (%)	COPD (%)	Asthma (%)	Control (%)
Male (n=65)	94.7	82.4	90	81%
Female(n=41)	100	100	63	85.7
Age gr.20-40 (n= 85)	97.3	100	80	88.9
41-60 (n=51)	93.8	80	70	73.3
> 60 (n=12)	100.0	83	100	-------

Distribution of H. pylori antigens by anti H. pylori IgG Immunoblot test

Sera from H. Pylori in lung inflammatory diseases were also analyzed using commercially available immunoblot strips coated with various anti H. Pylori antigens (EUROIMMUN).Control group individuals did not respond to any of the antigens. Patient group reacted to most of the antigens on the antigen coated immuno blot strips. Treatment groups under various age groups showed positivity in western blot. Results are shown in Table 2.

Table2: Anti *H. pylori* IgG immunoblot Test

H. Pylori Antigen	Tuberculosis (%) (N=23)	COPD (%) (N=21)	Asthma (%) (N=20)	Control (%) (N=20)
P120,cagA	78.3	71.4	75.0	85
P95,vacA	17.4	4.8	5.0	5
p75	17.4	0	5.0	5
P67,Flag	47.8	57	50.0	60
P66,ureB	47.8	61.9	55.0	70
P57	91.3	85.7	70.0	75
P54	52.2	66.7	30.0	45
P50	73.9	47.6	70.0	65
P41	47.8	38.1	60.0	55
P33	8.7	0	10.0	20.0
P30	30.4	9.5	0	25.0
P29,urea	52.2	42.9	15.0	45.0
P26	26.1	42.9	10.0	45
P19,omp	26.1	14.3	0	15.0
P17	4.3	0	0	5

Generating IL-10 Promoter SNP alleles by PCR

Important SNPs -1082(G/A), -819(C/T) and -592(C/A) located in the promoter region of IL-10 were studied in seropositive H. Pylori control populations and sero positive H. Pylori patients with respiratory diseases-TB, COPD and Asthma. SNP alleles analysis at -1082,-592,-819 was investigated if it could reveal significant associations with IL-10 production in lung related inflammatory diseases. Results are shown in Table 3. The results clearly indicate a close association of these SNPS with respiratory diseases in association with H. Pylori infection.

Table 3: IL-10 allele expression (%) among controls and patients with respiratory disorders.

Group/Disease	IL-10 (-592) (%)	IL-10 (-819) (%)	IL-10 (-1082) (%)
Group I TB(n= 50)	42.0	34.0	62.0
Group II COPD(n=27)	51.7	37	25.9
Group III Asthma (n= 21)	38.1	38.1	28.6
Group IV Control (n=42)	0.0	0	0

DISCUSSION

The micro flora of the gut are thought to modulate non-infectious diseases and are a major regulator of the immune system modulating inflammatory disorders and responses to infections.[11,12] This study investigated the inflammatory response to *H. pylori* infection, a common microflora in respiratory diseases like COPD, Asthma and Tuberculosis and association of IL-10 expressions in these inflammatory lung diseases.[13,14]

Production of interleukin-10 is regulated at translational and transcriptional levels. Different polymorphisms have been found in the 5 regions of the human interleukin 10 gene.[15,16,17] They include two repeat microsatellite polymorphisms and three point mutations at –1082 [G/A], –819 [C/T], and –592 [C/A]. The interleukin 10 genotype and functional response are related. The –1082 [G] point mutation is associated with increased production of interleukin 10 in T cells and monocytes, suggesting the critical role of this mutation in inflammatory responses.[18,19]

It was observed that Cag A antigen was prevalently expressed in COPD patients of the study cohort. Presence of H. pylori and particularly cytotoxin-associated gene A (CagA) antigens in COPD patients stimulate the release of a variety of proinflammatory cytokines, including IL-1, IL-8 and tumor necrosis factor. The underlying mechanisms, which induce and control this inflammatory process in COPD, are still unclear.[20,21] It is hypothesized that H. pylori infection might play a proinflammatory role and trigger COPD with other more specific environmental, genetic factors.IL-10 could also induce the production of IgE with IL-4[22] which is responsible for the allergeic response in asthma. Thus, IL-10 is suspected in asthma predisposition and the SNPs may have a genetic predisposition in triggering the cascade of adaptive immune response.

The H. pylori seroposivity in the TB group was significantly higher than that of controls. The serum concentration of IgG antibodies against H. pylori was also significantly higher in patients than in control subjects. It was observed in our study that H. pylori infection was associated with respiratory diseases along with significant interaction between various IL-10 SNPS and age. As intense inflammation is a key feature of H. Pylori infection, patients with a high pro-inflammatory condition were selected for the study. Among patients with TB and COPD, significant associations were found between the

IL-10 (-1082,-592) genotype. There was a significant interaction between age and the IL-10 polymorphism on the risk of respiratory diseases. Higher prevalence was observed in males within middle age group. The relative pro- versus anti-inflammatory response to infection differs with age. Patients in 30 yrs age group have expressed higher polymorphism of IL-10 at promoter site as evidenced by other reports on the increased IL-10 production in the inflammatory condition. There was a significant difference in the polymorphic sites of IL-10 SNPs in patients and controls. The IL-10 (-1082) prevalence was greater in TB patients as compared to IL-10 819(C/T) and -592(C/A) prevalence in asthma patients. Convincing evidence that certain of these polymorphisms-in particular the IL-10 -1082 SNP and associated IL-10, -819 and -592 haplotype are associated with differential expression of IL-10 in various H. Pylori associated respiratory diseases.[23,24]

CONCLUSION

The data conclusively suggests that there is an inherent genotype influence coupled with gene environment interaction in the inflammatory immune responses of respiratory diseases due to *H. pylori* infection.

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