Introduction

Inflammatory bowel disease is an idiopathic disease probably involving an immune reaction of the body to it is own intestinal tract. The two major type of IBD are ulcerative colitis and crohns disease as the name suggests, UC limited to the colon while CD can involve any segment of the gastrointestinal tract from mouth to the anus **(Rowe, 2007)**.

Despite all the advances in understanding the pathophysiology of IBD, it is exact cause remain unknown. The most widely accepted hypothesis is that overly aggressive acquired (T-cell) immune response to a subset to commensal enteric bacteria develop in genetically susceptible hosts, and environmental factor precipitate the onset or reactivation of the disease **(Sartor, 2006)**.

Under normal situation the intestinal mucosa is in the state of "controlled" inflammation regulated by a delicate balance of pro-inflammatory (tumor necrosis factor Alfa, interleukins, (1, 2, 6) and anti inflammatory cytokines (IL-4, IL-10, IL-11). Once the balance is disturbed, non specific stimulation and activation can lead to increase amount of potent destructive immunologic and inflammatory cells and molecules being activated and released with subsequent tissue destructive **(Ardizzone and Bianchi Porro, 2005)**.

Il-1 and TNF-Alfa increase the ability of epithelial cell, endothelial cell , macrophages and fibroblasts to secret potent chemotactic cytokines (IL-8,monocyte chemotactic protein-1) which serve to increase the movements of macrophages and granulocytes from the circulation to the inflamed mucosa **(Stroper et al., 2007)**.

The effect of TNF- Alfa are mediated by two specific receptors a 55-kDa protein (TNF receptor 1) and kDa-75 protein (TNF receptor 2), which are usually bounded to the cell surface.

Soluble TNF receptor 1 &2 are release by proteolytic cleavage of extra cellular domain of these receptors and acts as TNF antagonists that can inhibit TNF-Alfa mediated pro inflammatory activity **(Tanga et al., 2007)**.

An imbalance in secretion between TNF Alfa and TNFreceptor 1 may be implicated in the disease pathogenesis **(Noguchi et al., 1998)**.

Aim of the Work

The aim of this work is to study the balance between level of serum TNF-Alfa and serum soluble TNF-R 1 in patient with ulcerative colitis and the relation between serum soluble TNF R-1 and the disease activity state in these patient.

Chapter (1)
Ulcerative Colitis

Definition of IBD:

Inflammatory bowel disease is chronic relapsing and remitting inflammatory condition of the gastrointestinal tract which comprises ulcerative colitis and crohns disease **(Baumgart and Sandborn, 2007)**.

The pathogenesis of IBD still unknown, although in recent year more data has become available.

Both ulcerative colitis and crohns disease result from interrelated genetic and environmental factors that are channeled through an abnormality in mucosal immune function **(Langan, 2007)**.

Possibly due to dysregulated or excessive t-helper cell response. Strengthened by promising experimental data.

The pathophysiology of ulcerative colitis and crohns disease are quietly different. Namely, functional abnormalities of monocyte / macrophage and t- helper cell in crohns, while functional abnormalities of colonic epithelial cell and dysregulation of T / B cell suggested in ulcerative colitis **(Chande, 2007)**.

Definition of Ulcerative colitis:

Ulcerative colitis is chronic idiopathic inflammation of the rectal and colonic mucosa. There is possibility that ulcerative colitis might be a sort of syndrome **(Langan, 2007).**

Some might be viral, bacterial infection and some might be autoimmune. However, according to the evidence, the central core of ulcerative colitis is thought to be an organ specific autoimmune disease accompanied by many extra – colonic manifestations.

The autoantibodies against colonic epithelial cell (anticolon antibody) are frequently found in the serum of ulcerative colitis patient. These antibodies have been to shown to contribute to the destruction of colonic mucosa through antibody dependent cell mediated cytotoxicity mechanism against colonic epithelial cells to determine the specific antigen on colonic epithelial cell **(Mallon, 2007).**

UC is a disease of cleanliness. In common with diseases such as asthma, multiple sclerosis, and rheumatoid arthritis, it demonstrates an inverse relationship with the degree of sanitation: poor sanitation appears to protect against UC. The propensity for infection associated with overcrowding may also be a factor. It is postulated that improved hygiene alters the intestinal flora by decreasing exposure to certain critical

bacteria. There is an increased frequency of UC in higher socioeconomic groups (**Krishnan and Korzenik, 2002).**

Epidemiology

The peak age of onset for UC is 15 to 30 years old, although it may occur at any age. About 10% of cases occur in individuals <18 years old. UC has a bimodal age distribution, with a second, smaller peak occurring in individual's ages 50 to 70 years. Ulcerative colitis is slightly more common in males tend to occur in higher socioeconomic groups **(Andres and Friedman, 1999).** Breakdowns by racial and ethnic subgroups indicate that higher rates of UC occur in people of Caucasian and Ashkenazic Jewish origin than in individuals from other backgrounds. The distribution of UC among ethnic and racial groups remains dynamic. In past decades, it was thought that UC occurred less frequently in ethnic or racial minority groups compared with whites. This gap has been closing, with an increased incidence in African Americans and in second-generation south Asians who have migrated to developed countries **(Loftus and Sandborn, 2003)**.

Etiology

It is likely that a number of factors contribute to the development of mucosal inflammation. Also, variations in influence may account for the clinical diversity seen in UC. For example, a single family may have multiple affected members,

suggesting heightened genetic susceptibility. In contrast, sporadic disease, which accounts for the majority of UC cases, is more likely to be engendered by a unique environmental trigger or by a more subtle abnormality within the enteric immune system. Current etiologic theories concerning UC focus on environmental triggers, genetic factors, and immunoregulatory defects and microbial exposure **(Abreu, 2002).** So factors concerned with ulcerative colitis development include:

I. Environmental Triggers

A. Westernization

UC is most prevalent in developed regions, including the United States, United Kingdom, and Scandinavia **(Loftus and Sandborn, 2003).** The higher incidence of UC seen in industrialized countries and the dramatic increase in cases during the 20th century support the theory that environmental factors contribute to disease development **(Sandler and Loftus, 2004).** This may also account for the north-to-south variation and higher frequency in urban communities compared with rural areas. Interestingly, increases in incidence have recently been noted in southern countries and Asia and among migrants to first-world countries. It is postulated that this is the result of " westernization" of lifestyle, such as changes in diet, smoking,

and variances in exposure to sunlight, pollution, and industrial chemicals **(Loftus and Sandborn, 2003).**

B. Occupation

Higher mortality from UC has been noted in managerial, clerical, and sales positions, which typically involve sedentary, indoor work. In contrast, mortality resulting from UC is low among farmers and construction workers **(Sandler and Loftus, 2004). Sonnenberg** suggests that employment involving outdoor air and physical activity is protective against IBD, whereas work in artificial venues confers an increased risk (**Sonnenberg, 1990).** This theory could explain the higher risk for IBD in northern climates (e. g., more indoor exposure) and in immigrants to developed countries, as well as the varying rates among ethnic groups in different regions **(Andres and Friedman, 1999).**

C. Diet

Studies seeking to link diet and UC are generally inconclusive. There is some evidence that a higher intake of fatty acids increases the risk for UC (**Krishnan and Korzenik, 2002).** Similarly, **(Persson et al., 1992)** suggests that frequent fast-food intake confers a 3- to 4-fold greater risk for UC.

D. Tobacco smoking

The relationship between UC and smoking is complex, Numerous case-control studies have shown that current smoking is protective against UC (relative risk, 40% of that of nonsmokers), with results that are consistent across diverse geographic regions **(Sandler and Loftus, 2004).** The decreased risk for UC in smokers appears to be dose dependent. Current smoking also is protective against sclerosing cholangitis and pouchitis. Paradoxically, ex-smokers are approximately 1. 7 times more likely to develop UC than those who never smoked. Ex-smokers also have a poorer disease course, with more frequent hospitalization than current smokers; as a group they are twice as likely as current smokers and those who have never smoked to require colectomy **(Merrett et al., 1996).**

II. Familial and ethnic syndromes

There is an increased prevalence of UC in first- and second-degree relatives and a higher relative risk among siblings. The familial frequency of UC ranges from 20% to 30% in referral-based studies and between 5% and 10% in population surveys. The higher risk for IBD in the Jewish population suggests that genetic factors may play a larger role in some subgroups **(Satsangi et al., 2003).** In families with a high incidence of IBD among first-degree relatives, 75% of those affected are concordant for either UC or CD, whereas

25% are not concordant, with some members having UC and others having CD **(Binder, 1998).** This finding indicates that

III. Genetic Factors

Epidemiological and family studies demonstrate that genetic factors play a role in the susceptibility to UC**.** The disease is however, genetically complex and cannot be explained by a single gene model alone. It is thought that UC may be heterogeneous polygenic disorders sharing some but not all susceptibility loci. Most likely, the disease phenotype is determined by several factors, including interaction between allelic variants at a number of loci, as well as genetic and environmental influences. Consequently, the presence of a mutated gene does not guarantee that UC will develop, nor does it predict who will develop it, underscoring the importance of cofactors in precipitating the disease **(Satsangi et al., 2003).** Multiple, overlapping genetic factors may contribute to disease pathogenesis. Further support for a genetic susceptibility comes from the finding of an association between IBD and other syndromes with a genetic predisposition **(Satsangi et al., 2003).** Heritability studies indicate that there is a higher rate of concordance in monozygotic versus dizygotic twins for UC. For UC, reported concordance rates for monozygotic and dizygotic twins ranges from 6% to 17% and 0% to 5%, respectively, which is about the same as for non twin siblings **(Bouma and Strober, 2003).**

GENETIC BACKGROUND OF ULCERATIVE COLITIS

In the past decade, more than 10 genome-wide screening and various linkage studies have delineated at least nine IBD susceptibility loci (IBD1–IBD9). Many independent studies have shown that the NOD2/CARD15 polymorphism is not linked to ulcerative colitis, whereas Crohn's disease susceptibility is increased in European and American Caucasian carriers of the NOD2/CARD15 polymorphism **(Silverberg et al., 2005).** Nevertheless, several other genes have been studied as candidate loci linked to ulcerative colitis. Experimental studies have shown that multidrug resistance gene 1 (MDR1)-deficient mice develop colitis. Additional clinical studies showed that two polymorph (C3435T and G2677T/C) of the MDR1 gene are associated with ulcerative colitis. The human MDR1 codes for a P-glycoprotein that constitutes a barrier against xenobiotics. Polymorphism of this gene causes lower protein expression, and seems to be crucial in the defense against intestinal bacteria **(Schwab et al. 2003).**

However, other case–control studies did not confirm this finding. In the near future, outcomes of some ongoing studies on the IBD3 and IBD6 loci are expected. IBD3, located on chromosome 6p, contains the major histocompatibility complex genes. According to preliminary results, human leukocyte antigen alleles relevant to IBD seem to differ among ethnic groups (HLA-DRB1*0103). The IBD6 locus encompasses the

gene encoding integrin-binding membrane protein, which is crucial for immune cellular adhesion and trafficking **(Ahmad et al., 2003).**

The close association between the polymorphism of members of the nuclear factor κB (NFκB) family and IBD has recently been described **(Vermeire et al., 2004).** This linkage study, focused on the polymorphism of the promoter region of the human NFκB1 gene on chromosome 4q (the most prominent member of NFκB family), which is involved in a variety of regulatory processes (including innate and adaptive immunity, cellular growth, apoptosis and cell differentiation), has also been carried out in patients with ulcerative colitis **(Borm et al.,. 2005).** It was found that an increased frequency of the –94ATTG deletion polymorphism of the NFκB1 promoter in Dutch Caucasian patients with ulcerative colitis as compared with controls. Furthermore, homozygotic patients with a –94ATTG deletion were younger at onset of ulcerative colitis than non-homozygotic patients. The exact mechanism underlying the NFκB1-related disease susceptibility to ulcerative colitis remains unknown. One explanation might be a poor innate immune response to bacterial antigens owing to the low level of transcriptional proteins, leading to an invasion of the bacterial strains into the mucosa and the induction of chronic inflammation. Also currently being studied intensively are the genes encoding TLR4 and TLR9 that modify

responsiveness to intraluminal antigens in the gut **(Franchimont et al., 2004).**

IV. Role of the intestine's bacterial contents in ulcerative colitis.

Some of the similarities between ulcerative colitis and infectious colitides have led many investigators to search for the unidentified microorganism triggering the chronic inflammation in the large bowel. However, until now, no single microbial agent has been associated, unequivocally, with the development of ulcerative colitis **(Guarner, 2003)**.

Over the past few years, evidence suggests that it is an abnormal mucosal immune reactivity, against enteric bacteria, that is the key event leading to intestinal injury in patients with IBD. Molecular biology techniques have shown that the intestinal space of an adult may contain >500 different bacterial species; some of them exert a protective role, whereas others are aggressive. The number of bacterial strains along the small bowel progressively increases, with the predominance of Gram-negative aerobes. The bacterial population in the large bowel reaches a density of around 10^{12} microbes per gram of luminal contents. More than 50% of the bacterial strains cannot be cultured under conditions currently available. In adults, the fecal bacterial composition is host specific and stable over time,

with small fluctuations of the strains up to 20% **(Swidsinski et al., 2003)**.

The gut bacteria have an essential role in the development of the gut immune system, as they stimulate the lymphocytes to clonal expansion and also prevent lymphocyte apoptosis **(Marteau et al., 2004).** Selective bacterial stimulation may occur, with Gram-positive bacteria preferentially stimulating interleukin (IL)12 production, whereas Gram-negative organisms induce IL4 production **(Sartor, 2004).**

Although standard cultivation techniques are capable of detecting up to 30% of total microflora, new techniques (including analysis of bacterial 16S ribosomal RNA, polymerase chain reaction (PCR), in situ hybridization, flow cytometry and DNA microarray or chip analysis) have markedly increased the detection rate. The beneficial bacterial strains, such as bifidobacteria and lactobacilli, are generally absent from mucosa-associated bacterial flora in patients with active ulcerative colitis **(Kleessen et al., 2002).** On the other hand, an increased mucosal concentration of Gram-negative anaerobes, particularly Escherichia coli, Fusobacterium varium and bacteroides, along with a high frequency of Peptostreptococcus invasion, has been shown. Various authors have also shown severe bacterial invasions of the mucosa in

most colonic specimens from patients with ulcerative colitis, contrary to that in healthy controls **(Macfarlane et al., 2004).**

The high bacterial mucosal invasion in patients with IBD corresponds well with titers of immunoglobulin G to bacterial antigens. Some of these can now be used for distinguishing between ulcerative colitis (eg, anti-Peptostreptococcus anaerobius antibody) and Crohn's disease (eg, anti I2-from Pseudomonas fluorescens antibody or antibody to an outer membrane porin of E coli—anti-OmpC). Nevertheless, these differences in bacterial mucosal concentrations between ulcerative colitis and Crohn's disease were not found by several investigators **(Mow et al., 2004).**

The determination of our intestinal flora was previously proposed to be partially under genetic control. Changes in the fecal flora were also found among healthy relatives of patients with IBD. However, the question of whether the dysbiosis in patients with ulcerative colitis is the cause or the consequence of the disease still lacks a satisfactory answer **(Okhusa et al., 2002).** The role of intestinal bacteria in the etiopathogenesis of ulcerative colitis can be summarized as follows: Microbial flora in patients with ulcerative colitis differs considerably from that in controls, in both composition and spatial distribution (mucosal invasion) **(Swidsinski et al., 2003).** Some commensal bacterial strains exert an essential role in mucosal homeostasis