T. R.

KAHRAMANMARAŞ SÜTÇÜ İMAM UNIVERSITY

GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

INVESTIGATION OF *Val109/Asp* POLYMORPHISM OF OMENTIN GENE IN ULCERATIVE COLITIS PATIENTS

SAKAR AHMED ABDULLAH ABDULLAH

MASTER THESIS
DEPARTMENT OF BIOENGINEERING AND SCIENCES

KAHRAMANMARAŞ - TURKEY 2016

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M.Sc. thesis entitled “INVESTEGATION OF *Val109/Asp* POLYMORPHYSIM OF OMENTIN GENE IN ULCERATION COLITIS PATIENTS” prepared by Sakar Ahmed Abdullah ABDULLAH who is a student at Bioengineering and Sciences Department, Kahramanmaraş Sütçü İmam University, was certified 18/08/2016 by all of jury members, whose signatures are given below.

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I declare and guarantee that all information in this thesis has been obtained and presented in accordance with academic rules and ethical conduct. Based on these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

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ÜLSERATIF KOLIT HASTALARINDA

OMENTIN GEN *Val109/Asp* POLİMORFİZMİNİN İNCELENMESİ

(YÜKSEK LİSANS TEZİ)

SAKAR AHMED ABDULLAH ABDULLAH

ÖZET

Otoimmün hastalıklar, hastalıkların büyük ve kompleks bir grubunu oluştururlar ve hem genler hem çevre bu hastalıkların gelişiminde katkıda bulunur. Ülseratif kolit (ÜK) nedeni bilinmeyen otoimmün bir hastalıktır ve inflamatuar bağırsak hastalığı (IBD) spektrumuna aittir. ÜK heterojen bir hastalıktır ve birçok gen ÜK patogenezinde yer almaktadır. Omentin gen, yeni keşfedilen bir sitokindir. 8 ekson ve 7 introna sahiptir, ve kromozom 1 q22-23 de bulunur, Omentin'in bir anti-inflamatuar sitokin olmak gibi önemli rolleri vardır. Bugüne kadar omentin ve birçok enflamatuar hastalık (tip2 diyabet, CAD, RA, psoriasis gibi) arasındaki ilişki araştırılmıştır. Serum omentin-1 seviyesi pro-inflamatuar sitokin TNF-a, IL-6 düzeyleri ve CRP arasında negatif bir korelasyon mevcuttur. Bu çalışmada biz Polimeraz Zincir Reaksiyonu-Restriksiyon Parça Uzunluk Polimorfizm (PCR-RFLP) yöntemini kullamarak omentin genin Tek Nükleotid Polimorfizmi (SNP) ile ülseratif kolit hastalığı arasındaki olası ilişkiyi incelemeyi amaçladık. Val109/Asp (rs2274907) polimorfizmi yanlış anlamlı (missense) bir mutasyondur ve Omentin geninin ekson 4'ünde bulunmaktadir. Bu amaçla, biz 39 ÜK hastası ve 31 ÜK hastası olmayan bireyi kontrol grubu olarak kullandık. Her iki grup yaş ve cinsiyet yönünden benzerlik göstermekteydi. Veriler üzerinde gerçekleştirilen ki-kare testine göre, çalışılan genotip ile hastalık durumu arasında anlamlı bir ilişki olmadığı saptandı ($p=0.652$). Ancak, göreceli olasılıklar oranı (OR) hesaplandığında, TT genotipinin hasta grubunda kontrol grubuna göre 4.35 kat daha fazla görüldüğü tespit edilmiştir.

Anahtar Kelimeler: Omentin, ülseratif kolit, Val109/Asp SNP

Kahramanmaraş sütçü İmam Üniversitesi

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INVESTIGATION OF *Val109/Asp* POLYMORPHISM OF OMENTIN GENE IN ULCERATIVE COLITIS PATIENTS

(M. Sc. THESIS)
SAKAR AHMED ABDULLAH ABDULLAH

ABSTRACT

Autoimmune diseases are a large complex group of diseases, where both genes and environment contribute to disease development. Ulcerative colitis (UC) is an autoimmune disease of unknown etiology belongs to the spectrum of inflammatory bowel diseases (IBD). UC is a heterogeneous disease of which several genes have been involved in the pathogenesis. Omentin, is a newly discovered cytokine, has 8 exons and 7 introns it is locate at chromosome 1 q22-23. Omentin acts as an anti-inflammatory cytokine and the association between omentin and several inflammatory diseases such as (type2 diabetes, CAD, RA, psoriasis) has been investigated. In this study we aimed to investigate putative relationship between SNP (Single Nucleotide Polymorphism) in omentin gene and ulcerative colitis disease by using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method. Val109/Asp (rs2274907) polymorphism is a missense mutation (nonsynonymous substitution) located in exon 4 of the omentin gene, . For this aim, 39 patients diagnosed with UC and 31 cases (control) with no diagnosis of UC for were used. Both groups were at similar range of age and gender enrolled in our study. According to chi-square test run on the data, there was no significant correlation between the genotypes studied and the status of the disease (p=0.652). However, when odd ratios (OR) were calculated, it was determined that TT genotype was observed in patients as 4.35 times higher as observed in the control group.

Keywords: Omentin, Ulcerative colitis, Val109/Asp SNP

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Sakar Ahmed Abdullah Abdullah

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LIST OF ABBREVIATIONS

AST	**:**	Aspartate Aminotransferase
SNP	**:**	Single Nucleotide Polymorphism
AMPK	**:**	5′-AMP-activated Protein Kinase
AP1	**:**	Activator Protein-1
ALT	**:**	Alanine Aminotransferase
ALP	**:**	Alkaline Phosphatase
AID	**:**	Anti-inflammatory Diet
ANCA	**:**	Anti-Neutrophil Cytoplasmic Antibodies
ASCA	**:**	Anti-Saccharomyces Cerevisiae Antibodies
Asp	**:**	Aspatic Acid Amino Acid
AUD	**:**	Autoimmune Disease
Bp	**:**	Base Pair
Bpm	**:**	Beats Per Minute
BUN	**:**	Blood Urea Nitrogen
BMI	**:**	Body Mass Index
CRP	**:**	C-Reactive Protein
CVD	**:**	Cardiovascular Disease
JNK	**:**	C-Jun-N-terminal kinase
CD4+	**:**	Cluster of Differentiation 4+
CD8+	**:**	Cluster of Differentiation 8+

CSFs	**:**	Colony Stimulating Factors
cDNA	**:**	Complementary DNA
CBC	**:**	Complete Blood Count
CHD	**:**	Coronary Heart Disease
CRH	**:**	Corticotropin-releasing Hormone
Cre	**:**	Creatine
CD	**:**	Crohn's Disease
COX-2	**:**	Cyclooxygenase-2
DC	**:**	Dendritic Cell
D. Bil	**:**	Direct Bilirubin
eNOS	**:**	Endothelium Nitric Oxide Synthase
ESR	**:**	Erythrocyte Sedimentation Rate
FABP	**:**	Fatty Acid Binding Proteins
FFA	**:**	Free Fatty Acid
GGT	**:**	Gamma-glutamyl transpeptidase
Gly	**:**	Glycine
g/dL	**:**	gram/deciliter
Ht	**:**	Hematocrit
HDL	**:**	High-density Lipoproteins
HFD	**:**	High-Fat Diet
HLA	**:**	Human Lymphocyte Antigen

iNOS	**:**	Inducible Nitric Oxide Synthase
IBD	**:**	Inflammatory Bowel Disease
IkB	**:**	Inhibitor Nuclear Factor kappa-B
IFN	**:**	Interferons
IL	**:**	Interleukin
IL23R	**:**	Interleukin-23 Receptor
kD	**:**	Kilodalton
LDL	**:**	Low-density Lipoprotein
FODMAP	**:**	low–fermentable oligosaccharide, disaccharide, monosaccharide, and polyol
M1	**:**	Macrophage 1
M2	**:**	Macrophage 2
MHC	**:**	Major Histocompatibility Complex
MEC	**:**	Medullary Epithelial Cells
mRNAs	**:**	Messenger RNA
MetS	**:**	Metabolic Syndrome
µl	**:**	Microlitter
mg/h	**:**	milligram/hour
mm/h	**:**	Millimeter/hour
MCP-1	**:**	Monocyte-Chemoattractant Protein-1
NADPH	**:**	Nicotinamide Adenine Dinucleotide Phosphate-Oxidase

NF-κB	**:**	Nuclear Factor kappa-B
OR	**:**	Odds Ratio
PAT	**:**	Perivascular Adipose Tissue
PI3K	**:**	Phosphatidylinositol 3-Kinase
PAI-1	**:**	Plasminogen Activator Inhibitor type-1 PAI-1
PLT	**:**	Platelet Count
PCOS	**:**	Polycystic Ovary Syndrome
PCR-RFLP	**:**	Polymerase Chain Reaction-Restriction Fragment Length Polymorphism
PE2	**:**	Prostaglandin
Akt	**:**	Protein Kinase
ROS	**:**	Reactive Oxygen Species
RA	**:**	Rheumatoid Arthritis
SMC	**:**	Smooth Muscle Cell
SCD	**:**	Specific Carbohydrate Diet
SAPK	**:**	Stress-activated Protein Kinase
SAT	**:**	Subcutaneous Adipose Tissue
TCRs	**:**	T Cell Receptors
Th1	**:**	T-Helper Cell 1
Th17	**:**	T-Helper Cell 17
Th2	**:**	T-Helper Cell 2

Th	**:**	T-Helper Cell
TLR	**:**	Toll-like Receptor
T. Bil	**:**	Total Bilirubin
TGF-β1	**:**	Transforming Growth Factor
Treg	**:**	T-regulatory Cell
TGs	**:**	Triglycerides
TNF-α	**:**	Tumor Necrosis Factor-alpha
TNFR	**:**	Tumor Necrosis Factor Receptor
T2D	**:**	Type-2 Diabetes
UC	**:**	Ulcerative Colitis
UV	**:**	Ultra Violet
Val	**:**	Valin Amino Acid
VCAM	**:**	Vascular Cell Adhesion Molecule-1 VCAM
VAT	**:**	Visceral Adipose Tissue
WBC	**:**	White Blood Cell Counts

1. INTRODUCTION

Ulcerative colitis (UC) belongs to the inflammatory bowel disease (IBD) family. It's a chronic immunologically mediated inflammatory disorder of the gastrointestinal tract (Burisch, 2014) restricted to the colon and rectum (Paiotti et al., 2007). Etiology of ulcerative colitis disease remains uncertain (Choy et al., 1990). It's thought to be caused by the abnormal immune response and dysfunction of the intestinal mucosal barrier against enteric commensal bacteria in a genetically susceptible host (Burisch et al., 2013) triggered by the environment producing aggressive T cells that lead to the autoimmune response (Sartor, 2006).

Autoimmune diseases are a dysfunction of the adaptive immune system, and it leads the body to attack its own cells. Some genetic factors, environmental factors and infections may induce uncontrolled excessive immune responses to self-antigens by producing autoreactive T-lymphocytes (Liu, 2006, Patel, 2010).

Ulcerative colitis (UC) is diagnosed in late adolescence and early adulthood, and both genders are equally susceptible to Ulcerative colitis (Stange et al., 2008). Patients have a chronic intermittent disease flare with diarrhea, abdominal pain, bloody diarrhea, as well as mucus and pus per rectum which may require hospitalization. Intermediate with period of remission, UC is more common in industrialized countries with the highest incidence reported in the United Kingdom, and North America (Burisch, 2014).

Omentin, a newly discovered adipokine, have an anti-inflammatory role in inflammatory bowel disease (IBD) by preventing the tumor necrosis factor alpha (TNF-α) stimulated Cyclooxygenase-2 (COX-2) expression. Omentin is a visceral fat-specific adipokine expressed mainly in the visceral omental adipose tissue (Lu et al., 2014). Omentin gene expression is negatively correlated with C-reactive protein (CRP), low density lipoprotein (LDL), triglyceride levels, body mass index (BMI), obesity, blood pressure and insulin resistance while high density lipoprotein (HDL) level is positively correlated with the omentin plasma levels (de Souza Batista, 2004). Adipose tissue was considered as an organ that stores excess energy from triglyceride and release fatty acid when the system demands energy. In the last two decades, adipose tissue has been described as highly active endocrine organ and the major site producing most of pro-inflammatory and anti- inflammatory cytokines and hormones called adipokines to communicate with other organs including brain, liver, muscle, the immune system, and the

adipose tissue itself. The cytokines are a small secreted proteins that are involved in a the process of immunity, inflammation, and hematopoiesis by modulating gene expression and metabolic homeostasis (Pessin and Kwon, 2013).

Inflammation induced by obesity and metabolic disorder occurs by adipose tissue hypertrophy and hyperplasia by extra food intake inducing dyslipidemia leading to elevated plasma free fatty acid (FFA), triglycerides (TGs) and LDL with reduction of HDL. In turn, mitochondrial reactive oxygen species (ROS) are elevated thereby leading to mitochondrial damage due to lipid peroxidation and low expression of free radical detoxifying enzymes (Bjørndal et al., 2011, Yu, 2011). This metabolic imbalance changes expression some of adipokine gene such as omentin. Changing in omentin levels causes certain metabolic syndrome diseases (MetS) such as, ulcerative colitis, Crohn's disease, diabetes, obesity, coronary artery disease, polycystic ovary syndrome and skin lupus (Pessin and Kwon, 2013, Lu et al., 2014). Study by de Souza Batista et al. (2007) found that plasma level of omentin-1 and it's mRNAs are inversely correlated with obesity.

ROS such as superoxide anion is an important mediator in the pathogenesis of ulcerative colitis. Researchers observed superoxide anion producing cells in mucosal biopsy from patients with active IBD. ROS induce NF-κB in mononuclear cells then NF-κB induces pro-inflammatory cytokine TNF-α and inducible nitric oxide synthase (iNOS) expression (Dijkstra, 2004, Ramadori et al., 2015). Omentin, as an anti-inflammatory mediator, inhibits superoxide production by preventing the activation of p38 and c-Jun N-terminal kinase leading to inhibit TNF-α induced vascular cell adhesion molecule-1 (VCAM) expression (Lu et al., 2014).

Metabolic syndromes such inflammatory bowel disease caused by adipose tissue inflammation in genetically susceptible individuals or adipose tissue hypertrophy in obese persons lead to infiltration of immune cells due to the recruitment of T lymphocyte into the intestinal mucosa, thereby enhancing pro-inflammatory cytokine expression such as tumor necrosis factor (TNF-α) and certain interleukins including (IL-1, IL-6, and IL-12), while down-regulating anti-inflammatory cytokines such IL-10 (Bjørndal et al., 2011, Vázquez-Frias et al., 2015). TNF-α is an effective proinflammatory cytokine increased in active ulcerative colitis and have important roles in pathogenesis of UC by up-regulating Th2 and Th17 cells. While TNF-α up-regulate Th1 and Th17 cells in Crohn's disease, blockage of TNF-α by monoclonal antibodies treats active ulcerative colitis and Crohn's disease (Sartor, 2006). The anti-inflammatory and immune suppressive role of omentin by

inhibiting the TNF-α was approved by Zhong et al., 2012. They preincubated epithelial cells with 300 ng/ml omentin and then exposed them to TNF-α, expression of VCAM-1 mRNA was significantly suppressed compared with TNF-α treatment alone which strongly increased VCAM-1 mRNA. They also found that TNF-α directly phosphorylated ERK while omentin decreased of ERK phosphorylation indicating that omentin suppressed TNF enhanced ERK/NF-κB pathway thereby contributing to a significant increased of VCAM-1 mRNA expression in atherosclerosis patients. VCAM-1, as a biomarker of endothelial dysfunction, plays roles at the early stage of inflammatory response. It has been demonstrated that omentin observably acted as NF-κB inhibitor by inhibiting TNF-α induced degradation of IκB and NF-κB activation in cardio vascular diseases NF-κB is one of the most critical transcription factors contributed with inflammatory responses.

A balance between pro-inflammatory cytokine TNF-α and IL-6 and anti-inflammatory cytokine TGF-β1, and IL10 decide whether T cell differentiates to a Treg or a Th17 cell. T-helper cell 17 causes abnormal immune response against gut microbiota while the regulatory T cells have an important role in keeping the gut homeostasis by preventing the onset of the ulcerative colitis and termination immune response (Sartor, 2006, Bommireddy and Doetschman, 2007, Mockler et al., 2014, Kang et al., 2015). Pro-inflammatory cytokine TNF-α down regulate Treg differentiation while the anti-TNF-α maintains Treg-cell function in patients with severely active ulcerative colitis (Sandborn et al., 2012). The anti- TNF-α effect of omentin approved by Yamawaki et al. (2011) using recombinant human omentin pretreatment (300 ng/ml, 30 min) for patients with vascular diseases. The results demonstrated that omentin significantly inhibited the TNF-α induced COX-2 via preventing the JNK activation through activation of AMPK/eNOS/NO pathway. The AMPK activation by omentin may be through the phosphorylation of tyrosine kinase receptor. This clarifies the suppressing role of omentin on inflammation and suggests that the omentin may be used as a potential drug against inflammation.

Omentin is a 313-amino acid coded by 2 genes, omentin-1, which is the major circulating form, and omentin-2. Both omentin-1 and omentin-2 genes are localized adjacent to each other at a chromosomal region 1q22-q23 (Barth et al., 2010, Boron et al., 2015). Omentin premature mRNA has 8 exons and 7 introns (Turan et al., 2014). Studies have been shown that omentin mRNA expression and omentin plasma level are significantly decreased in the omental adipose tissue in ulcerative colitis and crohn's diseases compared to healthy control (Yin et al., 2015). One polymorphism in Omentin

gene is a nucleotide missense polymorphism in exon-4. It is a *Val109Asp* single nucleotide polymorphism (SNP), and it is an (A/T) polymorphism that results in replacement of the codon GAC by GTC. Consequently, the amino acid Asp at 109 changes to Val (Schäffler et al., 2007). Omentin has anti-inflammatory effects due to blocking TNF-α by suppressing JNK and NF-*κ*B in endothelial cells (Herder et al., 2013).

Our study is the first to investigate the association between omentin gene Val109Asp polymorphism and ulcerative colitis. Our approach depended on the previous researches which indicated that omentin plasma levels decreased during inflammatory diseases such as inflammatory bowel disease, cardiovascular disease, rheumatoid arthritis, asthma, and atherosclerosis and described omentin as an anti-inflammatory cytokine that suppress TNF-α, interleukin-6, interleukin-1, cyclooxygenase COX-2, and prostaglandin. We rationalized that UC, as an inflammatory disease, might have an association with omentin levels, which can be related to polymorphism carried on the omentin gene. In order to demonstrate this relation, we investigated Val109/Asp (rs2274907) single nucleotide polymorphism (SNP) in exon 4 among patients suffering from ulcerative colitis comparison with the age and gender matched individuals with no diagnosis of UC (control group) by using the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method. For this aim, 39 patients diagnosed with UC and 31 cases of the control group were included in our study. Genotypes were determined according to the bands formed in the agarose gel electrophoresis and the bands were visualized under the UV light. After collecting data of both groups, statistical analysis for genotypes were performed. First, chi-square test was run to find out whether there is a correlation between two variables. Then the odd ratios were calculated to determine the tendency of the genotypes in both groups.

2. LITERATURE REVIEW

2.1. Autoimmune Disease

Autoimmune diseases (AID) are dysfunction of both humoral and cellular immuniy in responses to tissue injury producing self-reactive lymphocytes which is aggressive against self-antigens then attack own healthy tissues in the body and damage it, Breaking down of immunological tolerance causes inefficiency to discriminate self-antigen from non-self (foreign) antigen producing an autoreactive immune cell (Lleo et al., 2010). Autoimmune diseases are complicated diseases caused by interaction between genetics, environmental factors, and epigenetics (Tobón et al., 2012). Autoimmune diseases are heterogeneous diseases including about 70 to 80 different diseases. Approximately 14.7 to 23.5 million people are affected in worldwide. The prevalence in the United States is about 3% to 8% of the population. Women have a remarkably higher risk of developing an autoimmune disease. Approximately 75% of autoimmune diseases patients are female due to up-regulating glucocorticoid level by estrogen female hormone whereas testosterone male hormone decreases glucocorticoid levels (Smith and Germolec, 1999, Dube et al., 2009). Autoimmune disease comprise several disorders and symptoms include organ-specific to systemic inflammatory disorders classified according to the target organ, tissue, and system (Dube et al., 2009). For instance, inflammatory bowel disease, rheumatoid arthritis, diabetes, and multiple sclerosis. Many of these diseases have the same symptoms with the cycles of exacerbation and remission. The first symptoms are fatigue with a low-grade fever and muscle aches, these diseases do not usually go away but symptoms can be treated (Smith and Germolec, 1999).

The immune system consists of lymphoid organs, lymph nodes, and immune cells (lymphocyte) with an intricate set of cellular, chemical, and protein components programmed to protect the body against foreign antigen without responding to self-antigen. Some soluble mediators such cytokines secreted by macrophage and lymphocytes have a role in the autoimmune disease pathogenesis (Smith and Germolec, 1999). Cytokines are proteins that promote cell-to-cell communication, including the tumor necrosis factors (TNFs), interleukins (ILs), interferons (IFNs), and transforming growth factors (TGFs). The effectiveness of these chemical information transporters depend on binding to specific receptors of the target cells surface (Kidd, 2003).

2.1.1. Innate and the adaptive immunity

Innate and the adaptive immunity are the two basic components of immune response. The innate immunity serves as the first line of defense responding immediately to nonspecific antigens. The system lacks the memory and removes the foreign substances by phagocytosis then recruit and activate T and B lymphocytes as an adaptive response (Ideström, 2012). This activation depends on recognizing antigens by lymphocytes which are presented by antigen presenting cells (APC). Dendritic cells are important antigen presenting cells. They take up antigens by phagocytosis. A section of the antigen become loaded by the major histocompatibility complex molecules (MHC) for presentation these antigens to T cells (Ercolini and Miller, 2009).

The fundamental role of immunity is that T cell lymphocytes attack foreign antigens but tolerate to self-antigen. The efficiency to differentiate self-antigen from non-self antigen is occurring in the thymus. The self-reactive cells in thymus producing an instant threat of autoimmunity are eliminated (Kronenberg and Rudensky, 2005, Lleo et al., 2010). Failure of self-tolerance causes immune responses to attach self-organ. Self-tolerance can be maintained by both central tolerance and peripheral tolerance (Bonifaz et al., 2002)

2.1.2. Central tolerance

Central tolerance occurs during differentiation of T cells in the thymus and differentiation B cells in bone marrow. T lymphocytes that recognize self-antigens undergo elimination by apoptosis (Bonifaz et al., 2002, Pearse, 2006). Thymus medullary epithelial cells (MECs) express tissues-specific antigens that are expressed in all cells outside the thymus. This ectopic expression has critical role in central tolerance. Defective thymic tolerance allow the escape of self-reactive T cells and development autoimmunity (Ohashi, 2003). Thymus controls differentiation of circulating T cells via positive and negative selection. The positive selection is major-histocompatibility complex MHC-restriction determined by the thymus cells. This restriction occurs first when naive T-cells are interacting with tissues-specific antigens in the thymus. Positive selection ensures that only T cells with TCRs that are able to recognize antigens presented with MHC I and II molecules will continue maturation (Pearse, 2006).

Negative selection stimulates death after the high-affinity interactions between T-cell receptors (TCRs) and self-antigens. Thymocytes with self- reactive T cell receptor

(TCR) become eliminatied by apoptosis along with the thymocytes with TCRs that do not interact with self-antige (Wang et al., 2001, Fiorini et al., 2002). Thymocytes with an intermediate affinity to self-antigen differentiated to CD25 called regulatory T cells (Treg), which down-regulate other immune cells and prevent autoimmunity. Thymocytes with low affinity to self-antigens survive (Smith and Germolec, 1999, Venanzi et al., 2004, Kronenberg and Rudensky, 2005). Positive selection confirms that double-positive thymocyte with a low affinity for self-antigen develops into CD4+ or CD8+ Single positive T cells, TCR restricted to MHC I differentiation into CD8+, is called cytotoxic T cells these cells, TCR restricted to MHC II differentiation into CD4+, is called helper T cells (Smith and Germolec, 1999, Sprent and Kishimoto, 2001, Pearse, 2006). T helper cells finally differentiate into Th1, Th2 cell, and Th17 depending on the cytokines they secreted. Thelper1 secret IFN-gamma and IL-12 while Th2 cells secrete IL-4. Over reactive Th2 can produce organ-specific autoimmune disease such as multiple sclerosis, arthritis, type 1 diabetes (Trembleau et al., 1995, Gumperz and Brenner, 2001, Kidd, 2003, Huang et al., 2012).

Thymus eliminates self-reactive T cells by apoptosis influenced by tumor necrosis factor (TNF) and glucocorticoids. (Akashi et al., 1997, Wang et al., 2001, Buch et al., 2002, Ohashi, 2003), apoptosis in negative selection is also induce by the c-Jun N-terminal kinase (JNK)/stress-activated protein kinase (SAPK) and p38/ mitogen-activated protein kinase (MAPK) pathway (Ohashi, 2003), while thymocyte survival during negative selection enhances inflammation by IL-7 promoted by phosphatidylinositol-3 kinase (PI3K) through PI3K/Akt pathway. Also transcription factors such as nuclear factor kB (NF-kB) influenced by some stimuli such as stress promotes thymocyte survival and differentiation (Fiorini et al., 2002, Ohashi, 2003).

2.1.3. Peripheral tolerance

Peripheral tolerance mechanisms are essential in case that central tolerance is imperfect. The mechanism controls the mature autoreactive lymphocytes that escape the central tolerance in thymus and migrate to the periphery and they are present within the peripheral lymphocyte pool. DCs in the periphery catch proteins from the airway and intestine and present these antigens (antigens that do not access the thymus) to T cells in lymphoid organs. Deletion of these potentially pathogenic autoreactive lymphocytes occurs via apoptosis through regulatory T-cells (Bonifaz et al., 2002, Kronenberg and Rudensky, 2005).

2.2. Ulcerative colitis

Ulcerative colitis is characterized by mucosal ulceration with a spectrum of chronic relapsing and remission course which is a subcategory of idiopathic inflammatory bowel disease with a lifelong inflammation that affects the rectum. It may spread in part or in the entire colon and cause ulceration and loss of function of the colon and rectum (Tian et al., 2003, Ideström, 2012). The etiology of this chronic immune disorder is still unclear (Shivananda et al., 1996). Therefore, the specific medical therapies are not yet available (Stange et al., 2008).

Inflammatory bowel disease (IBD) is a chronic relapsing inflammation of the bowel include both ulcerative colitis (UC) and Crohn's disease (Baumgart and Carding, 2007). As the name means, UC is continuous lesions limited to the colon and rectum that affects only the inner lining mucosal and submucosal layers (Huang et al., 2012). Ulceration involving only distal part of the colon and the rectum is called proctitis. Distal colitis is a disease of descending colon and ulcer cover the entire colon is called pancolitis (Figure2.1) (Head and Jurenka, 2003). In contrast, Crohn's disease is non-continuous and it can affect all part of the gut from mouth to colon and the whole thickness of the bowel wall (Huang et al., 2012).

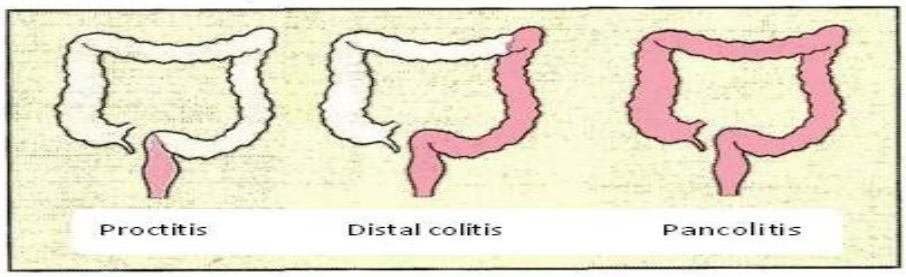


Figure2.1. Distribution of the ulcerative colitis disease in the different parts of the colon

Ulcerative colitis is characterized by producing aggressive T-cells in response of enteric bacteria. The onset of the disease is triggered by genetic abnormalities and environmental factors damaging the mucosal barrier and altering the balance between pathogenic enteric bacteria and beneficial bacteria (Sartor, 2006).

2.2.1. Symptomatology of ulcerative colitis

The primary prominent symptom of ulcerative colitis is bloody diarrhea, having been reported by more than 90% of UC patients (Stange et al., 2008). Ulcerative colitis either has a sudden onset. The first attack will be acute, rapid bursts and very aggressive,

or it may be an insidious onset that presents gradually abdominal cramping, flatulence, and diarrhea. When the disease progresses from mild to more severe, the symptoms may began to include bloody diarrhea, mucus in the stool, fatigue, weight loss, loss of appetite, severe rectal bleeding, fever, and anemia. Patients may also experience tachycardia, malnutrition, and mental abnormalities (Warren and Sommers, 1949, Head and Jurenka, 2003). The disease course is recognized by a period of a relapsing, with aggravation (flare-ups) followed by a spontaneous remission period for many years. The intensity of the relapses and the period of the remission vary between the individuals. Therefore the disease course is hard to predict (Ideström, 2012).

2.2.2. Disease stages

Patients with UC have a different extent of disease severity, disease stages is classified according to symptoms and sigmoidoscopy into remission, mild, moderate stage and severe stage (Truelove and Witts, 1955), as shown in Table2.1.

Complications	Mild	Moderate	Severe
Stool	<4	≥ 4	≥ 6
Tachycardia	<90 bpm	≤ 90 bpm	> 90 bpm
Temperature	<37.5 Cº	≤ 37.8 Cº	> 37.8 Cº
Hemoglobin	> 11.5 g/dL	≥ 10.5 g/dL	<10.5 g/dL
ESR	< 20mm/h	≤ 30mm/h	> 30mm/h
CRP	Normal	≤ 30mg/h	>30mg/h

Table2.1. The ulcerative colitis stages and clinically symptoms (Stange et al., 2008).

2.2.2.1. Severe colitis (active disease)

Severe colitis affects approximately 5 to 15% of ulcerative colitis patients, in the case of which hospital admission for intensive treatment is needed. Patient complications are severe diarrhea (six or more/day), blood in stools, fever that is over 37.8 ℃, anemia (hemoglobin 75% or less), Tachycardia the pulse rate will be more than 90/minute, and ESR will elevate to more than 30 mm/hour (Truelove and Witts, 1955). Abdominal distention will also be observed with tenderness and colonic dilation. When the colonic distention is more than 6 cm on radiography, it called toxic colitis or toxic megacolon, at this time surgery is required for 20-30% of patients. This occurs when medical therapy

fails and symptoms become uncontrollable despite of intensive medical regimen (Cohen et al., 2005).

2.2.2.2. Mild colitis

It is important to identify the stages of the disease since the treatment differs accordingly. Mucosal friability is the simplest clinical diagnosis to identify mild colitis from severe. Bleeding on simple contact with the rectal mucosa occurs during sigmoidoscopy in the severe case. The mild colitis symptoms are characterized by diarrhea (four or less/day), few blood in stools, anemia not severe, ESR below 30 mm/hour with no fever and no tachycardia (D'Haens et al., 2007).

2.2.2.3. Remission

Remission is recognized as a complete improvement of symptoms with mucosal healing, no fever, no tachycardia, one or two stools per day, stool without pus and blood, gaining weight, hemoglobin and ESR is normal or returning towards normal (Truelove and Witts, 1955).

2.2.2.4. Relapse

Over time, inflammation may progress or regress. Relapse is a flare of symptoms after remission stage. Rectal bleeding and increase stool frequency is an important indicator of relapse with abnormal mucosa at sigmoidoscopy (Stange et al., 2008).

2.2.2.5. Steroid-dependent colitis

It is the case where the patients are not able to reduce steroids treatment below the particular dose without experiencing a symptomatic relapse. It has been observed in the individuals whose disease remains asymptomatic only if they take steroids medication (D'Haens et al., 2007).

2.2.2.6. Refractory colitis

The case, when patients have active disease without improving in spite of receiving the medicine. Receiving Infliximab (anti-TNF-α antibody) is approved for patients with no response to conventional therapy (Rutgeerts et al., 2005).

2.2.3. Epidemiology of ulcerative colitis

Ulcerative colitis is often diagnosed in late childhood and early adulthood (Ideström, 2012). Prevalence of UC has remarkably increased since the early 1950s. The

highest incidences are common in more developed and industrialized countries. Until the last decade low prevalence have been reported from Asia and Eastern Europe, indicating that urbanization is a potential risk factor for UC pathogenesis (Shivananda et al., 1996, Lakatos, 2006). In Europe alone, more than 3 million individual are considered to suffer from IBD (Burisch, 2014).

2.2.4. Diagnosis of the ulcerative colitis

Ulcerative colitis diagnosis should be made by normal clinical, radiological, and pathological criteria.

2.2.4.1. Clinical details

Clinical diagnosis depends on abdominal tenderness, daily stool (frequency, amount of blood and consistency), pulse rate and temperature (Travis et al., 1996).

2.2.4.2. Laboratory investigations

The patients should have full laboratory tests including hematological tests (hematocrit, white blood count), biochemical tests (iron, albumin, creatinine), and inflammatory biomarker tests such CRP, ESR and liver function tests, Additionally, microbiological tests and culturing stool sample to differentiate symptomatic colitis from other infectious pathogens including *Clostridium difficile*, and *Escherichia coli* may be required. Laboratory signs may be normal during mild or moderate stages while elevated CRP, ESR, with anemia and hypoalbuminemia can be used as a biomarker for acute severe colitis indicating the need for colectomy (Stange et al., 2008).

Auto-antibodies have been used as a biomarker in UC diagnosis. Anti-neutrophil cytoplasmic antibodies (ANCA) is accurate biomarker for diagnose ulcerative colitis and titration of ANCA is used to measure severity of the disease (Kallenberg et al., 1994), whereas anti-Saccharomyces cerevisiae antibodies (ASCA) used as a biomarker for diagnosing crohn's disease (Sendid et al., 1998).

2.2.4.3. Radiology, Colonoscopy and Histopathology observation

Abdominal radiographs can be taken to observing presence of ulcer, and colonic dilatation (Travis et al., 1996).Whereas Colonoscopy can be used as a macroscopic diagnosis of disease. Taken mucosal biopsy is important for diagnosing the disease stages (Stange et al., 2008). The specimen should be taken from the most severely parts. Active disease could be diagnosed by the presence of neutrophils with damaged epithelial cell,

irregular surface and crypt abnormalities indicate mild disease while un modification of the lamina propria with lymphocyte infiltrate means quiescent disease (D'Haens et al., 2007).

2.2.5 Risk factors in the etiology of Ulcerative Colitis

Many factors trigger pathogenesis of UC such as genetic, immunologic factors, diet, environment, tobacco, infectious agents and the microbiome leading to a loss tolerance towards gut commensal bacteria Figure2.2 (Burisch, 2014).

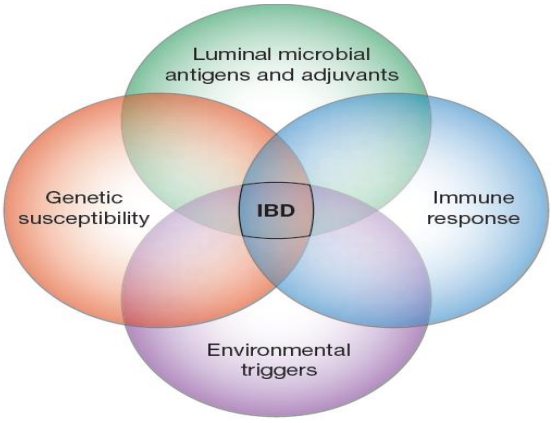


Figure2.2 Several factors contributing to chronic intestinal inflammation in genetically in genetically susceptible persons. (Sartor, 2006)

2.2.5.1. Diet

Studies have found interrelation between consumption of polyunsaturated fats and carbohydrates with inflammatory bowel disease, in Asia consumption of sugar is low, this is indicating the decreased incident of ulcerative colitis in Asia compared with America (Baumgart and Carding, 2007). Several diets have impact to relieve intestinal inflammation such as the specific carbohydrate diet (SCD), the anti-inflammatory diet (AID) and the (FODMAP) diet. The specific carbohydrate diet (SCD) depend on the theory that disaccharides and polysaccharides pass in the intestine without digestion until reaching the colon resulting overgrowth of bacterial and yeast, this dysbiosis cause inflammation. The western diet, with high in fat and protein that used now in worldwide is considered as indication for the increased incidence of IBD This diet includes limiting wheat, corn, barley, rice, milk products, sugar and all legumes are restricted (Olendzki et al., 2014, Knight-Sepulveda et al., 2015). A study by (Suskind et al., 2014) showed that 20 patients having ulcerative colitis and 31 patients having Crohn's disease receiving the SCD diet after 3 months 84% of the patients notes remission. The FODMAPs diet identified by a

highly fermentable carbohydrate but poorly absorbed by intestine, the rapid fermentation of these substances increase intestinal permeability (Knight-Sepulveda et al., 2015).

2.2.5.2. Vitamin D supplement

Vitamin D supplement could be a novel treatment option for IBD patients due to immunosuppressive role of Vitamin D, through reducing bacterial permeability to mesenteric lymph nodes and colonic lamina propria, increasing expression of the tight junction proteins, inhibition of T cells proliferation by inducing apoptosis through suppressing inflammatory cytokines such as TNF-α, IL-6, IL-17, suppressing Toll-like receptor (TLR), blocking NF-κB pathway, and switching T cell differentiation from Th17 toward a Treg cells. This is indicator of higher incidence of IBD with higher disease activity and longer disease duration in countries with lower exposure to sunlight such as northern Europe (Ryz et al., 2012, Parigi et al., 2015).

2.2.5.3. Smoking

Smoking have immunosuppressive effect and improve ulcerative colitis by inducing apoptosis of T-cells through AMPK pathway, decreasing VCAM and suppressing pro-inflammatory cytokines such as IL-1 and IL-6. UC predominantly affect on non-smokers and former smokers, increasing smoking dose decrease the disease severity, hospitalization and colectomy (Tysk et al., 1988, Regeling et al., 2016).

2.2.5.4. Genotyping

Several genes suspected to be involved in susceptibility to UC, the most implicated gene is HLA gene, the Interleukin-23 Receptor (IL23R) gene and Toll-like Receptor (TLR) gene. Study on twins and the first-degree relatives indicate the strong evidence that genetic factors are completely involved (Stoll et al., 2004, Stange et al., 2008).

2.2.5.5. Stress

Depression increase he relapse in patients by increasing colonic permeability of the mast-cell due to altered expression of tight junction proteins, verproduction of interferon-γ (Tysk et al., 1988, Baumgart and Carding, 2007). Female have higher stress hormone glucocorticoids enhanced by estrogen resulting in Th2 immune response, while testosterone male hormone suppress glucocorticoid expression. Childhood abuse was associated with increased risk to inflammation due to stimulating corticoid-releasing hormone (CRH) increasing glucocorticoids (Dube et al., 2009).

2.3. Adipose tissue

Adipose tissue previously was characterized to be a storage site for excess energy as a triacylglycerol, but in the past decade, adipose tissue described as an endocrine organ that secret bioactive molecules called adipokines (Galic et al., 2010). Adipose tissue consists of adipocytes and non-adipose cells (vascular-stromal fraction) including preadipocytes, macrophages, fibroblasts, and endothelial cells, both of adipocytes and vascular stromal fraction are sources of adipokines such as adiponectin and resistin involved in glucose metabolism, leptin hormone involved in feeding behavior, plasminogen activator inhibitor type-1 PAI-1 involved in coagulation, and (TNF-a and IL-6) cytokines involved in systemic inflammation (Hajer et al., 2008, Alissa et al., 2016). The major adipose depots are existing in the abdomen and are divided into the visceral adipose tissue and subcutaneous adipose tissue, Furthermore, the visceral tissue divided into omental and mesenteric adipose tissue (Bjørndal et al., 2011). Visceral adipose tissue implicated in obesity-related diseases like hypertension and atherosclerosis, as well as it is involved in secreting pro-inflammatory cytokines including (TNF-α, interleukin-6) and anti-inflammatory cytokine omentin and adiponectin (Kazama et al., 2012).

During obesity adipocytes release a massive amount of free fatty acid (FFAs) through lipolysis, the FFAs bind to the toll-like receptor-4 of macrophages enhancing macrophage to produce pro-inflammatory cytokine (TNF-α) through the NFκB and AP1 pathway (Preedy and Hunter, 2011) causes chronic inflammation and insulin resistance (McGuire and Marx, 2014). TNF-α induces the secretion of other proinflammatory adipokines by adipose tissue such as MCP-1, IL-6 and IL-1. MCP-1 enhance macrophage recruitment and infiltration to adipose tissue, thereby proinflammatory cytokines secreting by macrophage increase (Preedy and Hunter, 2011), also T-hepler cells were found to be infiltrated in adipose tissue while Treg cells levels reduced, leading to metabolic syndromes disease such as type 2 diabetes, vascular diseases. Study has been shown that high fat diet (HFD) and saturated fat worsen the ulcerative colitis through toll-like receptor-4 (TLR4) mediated immunity. Chronic weight loss reduces the macrophage levels while acute weight loss or fasting enhances elevation of free fatty acid (Hajer et al., 2008, Galic et al., 2010, Ota and Das, 2015).

2.4. Omentin

Omentin is recently identified as a novel circulating anti-inflammatory adipocytokine expressed by stromal vascular cells of visceral adipose tissue (Yamawaki et al., 2011), but not by adipocytes. It is also expressed in lung, heart, placenta, and ovary. Omentin circulating level is inversely correlated with insulin resistance, body mass index (BMI), and the metabolic syndrome (MS) while it is positively correlated with HDL and adiponectin (Wang, 2014, Kohan et al., 2016). Omentin has the protective roles in intestinal immune system against pathogenic bacteria such as *Escherichia coli* (Tan et al., 2015).

Omentin has an important role in modulating immune response. Down-regulating of omentin was shown to be implicated in several chronic inflammatory diseases such as coronary heart disease (CHD) (Wang et al., 2014), ulcerative colitis UC (Yin et al., 2015), polycystic ovary syndrome (PCOS) (Tan et al., 2010), insulin resistance, diabetes (Yamawaki et al., 2011), rheumatoid arthritis (Tan et al., 2015) and some other autoimmune disorders (Assadi et al., 2011).

2.4.1. Omentin gene

Omentin is a hydrophilic protein composed of 313 amino acids with molecular weight of 35 kDa mainly expressed in stromal-visceral adipose tissue. Omentin was initially found in intestinal cells called intelectin or lactoferrin receptor, and found in endothelial cells called endothelial lectin. Omentin was discovered and identified in omental adipose tissue cDNA library in 2003 (de Souza Batista et al., 2007, Assadi et al., 2011, Tan et al., 2015). Omentin was also detected in human serum by western blot analysis. There is a 100 % homology between the amino acid sequence of omentin and a intelectin protein which is able to recognize carbohydrate galactofuranose of bacterial cell walls (Schäffler et al., 2007). Intelectin plays an important role in chronic inflammation such as Crohn's disease and ulcerative colitis by defending against intestinal bacteria. The omentin protein has signal peptides consisting of 16-amino acid, and the putative cleavage site is located at Gly 16 Fiqure2.3 (Schäffler et al., 2005). After cleaving the signal sequence, mature omentin has 296 amino acids with a molecular weight of 33 kDa and circulating omentin protein plasma levels in humans are ranged between 1 ng/mL to 100 ng/mL (Preedy and Hunter, 2011).

ATG	AAC	CAA	CTC	AGC	TTC	CTG	CTG	TTT	CTC	ATA	GCG	ACC	ACC	AGA	15
Met	Asn	Gln	Leu	Ser	Phe	Leu	Leu	Phe	Leu	Ile	Ala	Thr	Thr	Arg	
GGA	TGG	AGT	ACA	GAT	GAG	GCT	AAT	ACT	TAC	TTC	AAG	GAA	TGG	ACC	30
Gly	Trp	Ser	Thr	Asp	Glu	Ala	Asn	Thr	Tyr	Phe	Lys	Glu	Trp	Thr	
TGT	TCT	TCG	TCT	CCA	TCT	CTG	CCC	AGA	AGC	TGC	AAG	GAA	ATC	AAA	45
Cys	Ser	Ser	Ser	Pro	Ser	Leu	Pro	Arg	Ser	Cys	Lys	Glu	Ile	Lys	
GAC	GAA	TGT	CCT	AGT	GCA	TTT	GAT	GGC	CTG	TAT	TTT	CTC	CGC	ACT	60
Asp	Glu	Cys	Pro	Ser	Ala	Phe	Asp	Gly	Leu	Try	Phe	Leu	Arg	Thr	
GAG	AAT	GGT	GTT	ATC	TAC	CAG	ACC	TTC	TGT	GAC	ATG	ACC	TCT	GGG	75
Glu	Asn	Gly	Val	Ile	Tyr	Gln	Thr	Phe	Cys	Asp	Met	Thr	Ser	Gly	
GGT	GGC	GGC	TGG	ACC	CTG	GTG	GCC	AGC	GTC	CAT	GAG	AAT	GAC	ATG	90
Gly	Gly	Gly	Trp	Thr	Leu	Val	Ala	Ser	Val	His	Glu	Asn	Asp	Met	
CGT	GGG	AAG	TGC	ACG	GTG	GGC	GAT	CGC	TGG	TCC	AGT	CAG	CAG	GGC	105
Arg	Gly	Lys	Cys	Thr	Val	Gly	Asp	Arg	Trp	Ser	Ser	Gln	Gln	Gly	
AGC	AAA	GCA	GAC	TAC	CCA	GAG	GGG	GAC	GGC	AAC	TGG	GCC	AAC	TAC	120
Ser	Lys	Ala	Asp	Tyr	Pro	Glu	Gly	Asp	Gly	Asn	Trp	Ala	Asn	Tyr	
AAC	ACC	TTT	GGA	TCT	GCA	GAG	GCG	GCC	ACG	AGC	GAT	GAC	TAC	AAG	135
Asn	Thr	Phe	Gly	Ser	Ala	Glu	Ala	Ala	Thr	Ser	Asp	Asp	Tyr	Lys	
AAC	CCT	GGC	TAC	TAC	GAC	ATC	CAG	GCC	AAG	GAC	CTG	GGC	ATC	TGG	150
Asn	pro	Gly	Tyr	Tyr	Asp	Ile	Gln	Ala	Lys	Asp	Leu	Gly	Ile	Trp	
CAC	GTG	CCC	AAT	AAG	TCC	CCC	ATG	CAG	CAC	TGG	AGA	AAC	AGC	TCC	165
Met	Val	Pro	Asn	Lys	Ser	Pro	Met	Gln	His	Trp	Arg	Asn	Ser	Ser	
CTG	CTG	AGG	TAC	CGC	ACG	GAC	ACT	GGC	TTC	CTC	CAG	ACA	CTG	GGA	180
Leu	Leu	Arg	Tyr	Arg	Thr	Asp	Thr	Gly	Phe	Leu	Gln	Thr	Leu	Gly	
CAT	AAT	CTG	TTT	GGC	ATC	TAC	CAG	AAA	TAT	CCA	GTG	AAA	TAT	GGA	195
His	Asn	Leu	Phe	Gly	Ile	Tyr	Gln	Lys	Try	Pro	Val	Lys	Tyr	Gly	
GAA	GGA	AAG	TGT	TGG	ACT	GAC	AAC	GGC	CCG	GTG	ATC	CCT	GTG	GTC	210
Glu	Gly	Lys	Cys	Trp	Thr	Asp	Asn	Gly	Pro	Val	Ile	pro	Val	Val	
TAT	GAT	TTT	GGC	GAC	GCC	CAG	AAA	ACA	GCA	TCT	TAT	TAC	TCA	CCT	225
Try	Asp	Phe	Gly	Asp	Ala	Gln	Lys	Thr	Ala	Ser	Tyr	Tyr	Ser	Pro	
TAT	GGC	CAG	CGG	GAA	TTC	ACT	GCG	GGA	TTT	GTT	CAG	TTC	AGG	GTA	240
Tyr	Gly	Gln	Arg	Glu	Phe	Thr	Ala	Gly	Phe	Val	Gln	Phe	Arg	Val	
TTT	AAT	AAC	GAG	AGA	GCA	GCC	AAC	GCC	TTG	TGT	GCT	GGA	ATG	AGG	255
Phe	Asn	Asn	Glu	Arg	Ala	Ala	Asn	Ala	Leu	Cys	Ala	Gly	Met	Arg	
GTC	ACC	GGA	TGT	AAC	ACT	GAG	CAC	CAC	TGC	ATT	GGT	GGA	GGA	GGA	270
Val	Thr	Gly	Cys	Asn	Thr	Glu	His	His	Cys	Ile	Gly	Gly	Gly	Gly	
TAC	TTT	CCA	GAG	GCC	AGT	CCC	CAG	CAG	TGT	GGA	GAT	TTT	TCT	GGT	285
Tyr	Phe	Pro	Glu	Ala	Ser	Pro	Gln	Gln	Cys	Gly	Asp	Phe	Ser	Gly	
TTT	GAT	TGG	AGT	GGA	TAT	GGA	ACT	CAT	GTT	GGT	TAC	AGC	AGC	AGC	300
Phe	Asp	Trp	Ser	Gly	Tyr	Gly	Thr	His	Val	Gly	Tyr	Ser	Ser	Ser	
CGT	GAG	ATA	ACT	GAG	GCA	GCT	GTG	CTT	CTA	TTC	TAT	CGT	TGA		313
Arg	Glu	Ile	Thr	Glu	Ala	Ala	Val	Leu	Leu	Phe	Tyr	Arg	*Ter*		

Figure2.3 Nucleotide sequence and amino acid sequence of the omentin gene. The 16 amino acid signal peptide is underlined, the cleavage site at Gly is colored green, (Tre) is the TGA stop codon, the SNP site ASP 109 colored yellow

2.4.2. Chromosomal region of Omentin

Omentin have two homologous genotypes and is encoded and expressed via 2 different genes. Omentin-1 and omentin-2 with 83% of amino acid similarity, omentin1 is the major circulating form in human plasma (Tan et al., 2015). Omentin 1 and Omentin 2 chromosomal regions are located adjacent to each other on the chromosome 1q22-q23 loci (de Souza Batista et al., 2007, Yoo et al., 2011). The human omentin gene contain of 8 exons and 7 introns. Exon 1 consists of 199 bp including 5′-untranslated region, the 5' region of the omentin gene contains putative binding sites for transcription factors involved in regulation of cytokine expression, exon 2 consists of 64 bp (containing 6 bases of 5′-UTR and the ATG start codon). Exon 3 consists of 99 bp, exon 4 of 248 bp, exon 5 of 159 bp, exon 6 of 121 bp, exon 7 of 104 bp and, exon 8 of 277 bp. The 3′-untranslated region consists of additional 100 bp. All exon/intron boundaries were splicing by cannonical AG/GT rule. Intron 1 consists of 177 bp, intron 2 consists of 1293 bp, intron 3 consists of 1223 bp, intron consists 4 of 644 bp, intron 5 consists of 445 bp, intron 6 consists of 1173 bp and, intron 7 of consists 2494 bp Figure2.4 (Schäffler et al., 2005).

2.4.3. Omentin Val109Asp SNP

There is a single nucleotide missense polymorphism (SNP) Val109Asp in omentin gene sequence occurs when (Asp) amino acid with codon GAC changed to (Val) amino acid with codon GTC by single nucleotide A/T changing at position 109 of omentin gene sequence, homozygote Asp/Asp (GAC/GAC) become homozygote mutant Val/Val with genotype (GTC/GTC) or heterozygote mutant Val/Asp with genotype (GTC/GAC) (Schäffler et al., 2007), changing in the genetic code will be a mutation or a polymorphism, an allelic variant that occurs at a frequency greater than 1/100 within a population is indicating as a polymorphism (Buckingham, 2011, Ideström, 2012)

Exon	Size	Splice Acceptor	Exon Sequence	Splice donor	Intron	Size
1	199 bp		GGCATTGTGCCAGGGGAGGGTGAGGCTGGAAACCTTGGT TGGCCCCACTGGGCTTCCTCCATAAAGCTTTCTGCACCT CATTCCACATCAGGAGCGTTTTTGGAGAAAGCTGCACTC TGTTGAGCTCCAGGGCGCAGTGGAGGGAGGGAGTGAAGG AGCTCTCTGTACCCAAGGAAAGTGCAGCTGAGACTCAGA CAAG	gtctgtgagt	1	197bp
2	64 bp	ctttctttag	ATTACA**ATG**AACCAACTCAGCTTCCTGCTGTTTCTCATA GCGACCACCAGAGGATGGAGTACAG	gtgagtcacg	2	1293 Bp
3	99 bp	tattccctag	AGAGGCTAATACTTACTTCAAGGAATGGACCTTGTTCTT CGTCTCCATCTCTGCCCAGAAGCTGCAAGGAAATCAAAG ACGAATGTCCTAGTGCATTTG	gtgagtgatg	3	1223 Bp
4	248 bp	ctctctacag	ATGGCCTGTATTTTCTCCGCACTGAGAATGGTGTTATCT ACCAGACCTTCTGTGACATGACCTCTGGGGGTGGCGGCT GGACCCTGGTGGCCAGCGTGCACGAGAATGACATGCGTG GGAAGTGCACGGTGGGCGATCGCTGGTCCACTCAGCAGG GCAGCAAAGCA**GTC**TACCCAGAGGGGGACGGCAACTGGG CCAACTACAACACCTTTGGATCTGCAAGGGCGGCCACGA GCGATGACTACAAG	gttggtgcca	4	644bp
5	159 bp	tgctttccag	AACCCTGGCTACTACGACATCCAGGCCAAGGACCTGGGC ATCTGGCACGTGCCCAATAAGTCCCCCATGCAGCACTGG AGAAACAGCTCCCTGCTGAGGTACCGCACGGACACTGGC TTCCTCCAGACACTGGGACATAATCTGTTTGGCATCTAC CAG	gtacagaagg	5	445bp
6	12Lbp	ttctttgtag	AAATATCCAGTGAAATATGGAGAAAGGAAAGTGTTGGACT GACAACGGCCCGGTGATCCCTGTGGTTCTATGATTTTGGC GACGGCCCAGAAAACAGCATCTTATTACTCACCCTATGG CCAGC	gtgagtcttt	6	1173bp
7	104 bp	ttgttttcag	GGGAATTCACTGCGGGATTTGTTCAGTTCAGGGTATTTA ATAACGAGAGAGCAGCCAACGCCTTGTGTGCTGGAATGA GGGTCACCGGATGTAACACTGAGCAC	gtgagtccct	7	2494bp
8	277 bp	tgtttccag	CATTGCATTGGTGGAGGAGGATACTTTCCAGAGGCCAGT CCCCAGCAGTGTGGAGATTTTTCYGGTTTTGATTGGAGT GGATATGGAACTCATGTTGGTTACAGCAGCAGCCGTGAG ATAACTGAGGCAGCTGTGCTTCTATTCTATCGT**TGA**	3'-UTR 100 BP		

Figure2.4. Exon and intron structur of the human omentin gene exon bases is capital letter, intorn bases in small letters, splicing site ag/gt of exon and intron , start codon ATG and TGA stop codon colord blue, SNPs site GTC at 328 bp colord green

2.4.4. Omentin plasma level

Study by (Auguet et al., 2011) showed that Omentin expression significantly decrease in visceral adipose tissue in overweight/obese individuals, also demonstrate that omentin mRNA expression and plasma levels are inversely correlated with increased glucose level and increased BMI. Recombinant omentin-1 enhances glucose uptake in omental adipose tissue through phosphorylation of Akt pathway. Omentin plasma level increase after administration with metformin which is diabetic therapy and improves insulin sensitivity through Akt phosphorylation pathway indicating that serum level of omentin is associated with weight loss (Wang, 2014). Study by (Wang et al., 2014) demonstrated that omentin plasma level decrease in coronary heart disease (CHD) compared with healthy subject.

The recent study by Abd-Elbaky et al. (2015) found that Omentin plasma levels were decreased significantly in diabetic patients compared to the healthy individual. Omentin plasma level considered to be correlated with diabetes, visceral obesity, and glucose metabolism. Also study by Lapointe et al. (2014) approved that Omentin plasma levels elevated after bariatric surgery and after weight loss improve cardiovascular function.

2.4.5 Omentin as an anti-inflammatory cytokine

Omentin has generate an anti-inflammatory effect by suppressing the tumor necrosis factor-α (TNF-α) inducing cyclooxygenase COX-2 expression through preventing the JNK signaling pathway in human endothelial cells (Yoo et al., 2011). A study by Maruyama et al. (2012) found the anti-inflammatory role of omentin by inhibiting TNF-α levels through phosphorylation of AMPK through AMPK/eNOS/NO pathway in the cultured human endothelial cells. Omentin activate p38 by phosphorylation that leads to inhibiting TNF-α-induced expression of VCAM-1 (Tan et al., 2015), p38 is contributed with inflammatory bowel by reducing inflammation through inhibiting expression of TNF, IL-6, COX-2, and VCAM Figure 2.5 (Zarubin and Jiahuai, 2005).

2.4.6. Omentin inhibit Reactive Oxygen Species (ROS)

Study by Kazama et al. (2012) showed that Omentin (300 ng/ml, for 30 min) inhibit reactive oxygen species induced by TNF-α in VSMCs, reactive oxygen species mediate inflammation during the oxidative burst in leukocytes (Maghzal et al., 2012, Ray et al., 2012). Study by (Onodera et al., 2015) approved that reactive oxygen species ROS mediate

inflammation by up-regulating of Cox-2 and prostaglandins (PGs) production due to activation of MAPK/P38/JNK pathway through transcriptional factor NF-kB or activator protein 1 (AP-1). ROS is also induce proliferation through PI3K/Akt pathway (Ray et al., 2012, Onodera et al., 2015).

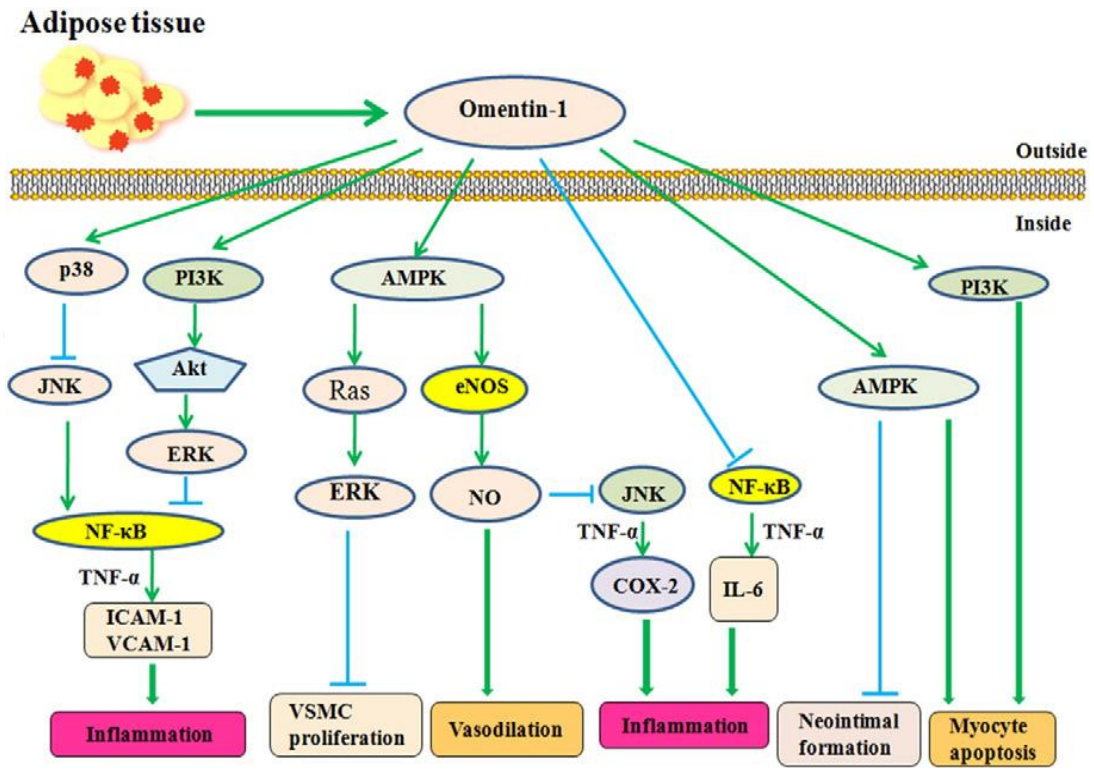


Figure2.5. Omentin's roles as anti-inflammatory cytokine by AMPK phosphorylation, and activates eNOS, which has vasodilation effect, omentin blocks JNK induced inflammation through TNF-α-mediated COX2. Moreover, omentin suppress the NF-κB signaling pathway and inhibits inflammation. The anti-inflammatory effect of omentin is through inhibiting Akt and NF-κB pathways. Omentin causes vasodilatation by increasing nitric oxide production via endothelial nitric oxide synthase (Tan et al., 2015).

2.4.7. Omentin enhances nitric oxide (NO)

Omentin expressed in epicardial adipose tissue (EAT) has hypotensive and vasoprotective roles by inhibiting TNF-α and IL-6 in macrophages through phosphorylation of endothelial NO synthase (NOS) by Akt/eNOS signaling pathway in isolated blood vessels. Decreasing circulating omentin level is regarded as a marker of endothelial dysfunction. (Brunetti et al., 2014, Wang et al., 2014, Kocijancic et al., 2016).

2.4.8. Omentin attenuated Cyclooxygenase (COX-2)

Study by (Yamawaki et al., 2011) discovered that pretreatment with omentin (300 ng/ml, 30min) decrease cyclooxygenase-2 by inhibiting the JNK through of AMPK/eNOS/NO pathway, COX-2 is inducible enzyme contributed with ulcerative colitis by up-regulating prostaglandins (PGs) synthesis. Glucocorticoids inhibit COX-2, while TNF-α and IL-1 enhance COX-2 through p38/JNK pathway (Keskek et al., 2006, Paiotti et al., 2007, Shibata et al., 2011, Onodera et al., 2015)

Study by (Singer et al., 1998) showed that COX-2 detected in isolated epithelial cells from ulcerative colitis patients while COX-2 was not detected in normal epithelial cells, also prostaglandin level remarkably elevated in ulcerative colitis patients. A study by (Boniface et al., 2009) showed that prostaglandin (PGE2) directly promotes Th17 cells differentiation by enhancing pro-inflammatory cytokine IL-17. Cyclooxygenase-2 (COX-2) inhibitors inhibit prostaglandin synthesis and reduce the severity of autoimmune disease.

2.4.9. Omentin suppress (TNF-α) preventing auto reactive T-cells

Study by (Tan et al., 2010) demonstrated that adding omentin-1 (200 ng/ml) to serum significantly suppress TNF- α promoting NF-κB activation , also adding omentin-1 (200 ng/ml) to serum significantly decrease activation of Akt indicating the anti-inflammatory effect of omentin in PCOS. Administration with recombinant omentin inhibits TNFα and decreases inflammation by activating AMPK/eNOS pathway (Wang, 2014). The stromal vascular fraction is the main source of adipose derived TNF-α from macrophages and Tcells (Galic et al., 2010, Sedger and McDermott, 2014).

LPS and reactive oxygen species promote TNF to enhance cell survival and produce inflammation through MAPK/JNK/p38 pathway, TNF-α is also enhance survival by transcriptional factor NF-kB, and activator protein AP1 enhancing expression of IL-6 and prostaglandins (Zarubin and Jiahuai, 2005, Sedger and McDermott, 2014). The study by (Campbell et al., 2004) showed that the activity of p38/MAPK resulted in inhibiting 30–50% TNF-α mRNA in rheumatoid arthritis synovial cells, TNF-a induces transcription of itself through MAPK/p38 pathway.

In the inflammatory bowel disease, luminal antigens accessing to the underlying mucosal tissue via a leaky lamina propria barrier this lead toll like receptor falsely recognize commensal bacteria or dietary as antigens and the antigens uptakes by APCs (Baumgart and Carding, 2007, Sedger and McDermott, 2014, Parigi et al., 2015). The

loaded APCs migrate into the lymph nodes (Peyer's patches) to present antigen and differentiation naïve T cells into effector T cells. Lymphocyte differentiation is controlled by the interaction of TNF-α, reactive oxygen species (ROS), interferon gamma (IFN-γ) and transforming growth factor TGF (Bonizzi and Karin, 2004, Parigi et al., 2015). Ulcerative colitis and Crohn's disease characterized by disturbed Tcell homeostasis and the effector Tcells (Th1, Th2) are predominate over regulatory T cells, in ulcerative colitis naive Tcells differentiate into Th2 secreting (IL-5, and IL-4) while in Crohn's disease naive Tcells (Th0) differentiate into Th1 cells secreting (interferon-γ, and interleukin 12). The Th17 cells secrete inflammatory cytokine IL-17 maintained by IL-23 are contributed to develop both ulcerative colitis and Crohn's disease while regulatory T-cells secreting anti-inflammatory cytokine (TGF, IL10) decrease in both diseases (Baumgart and Carding, 2007, Cătană et al., 2015). TNF-α have important role on differentiation naïve Tcells form Treg to Th17, Th1 and Th2 in inflammatory bowel disease producing autoreactive T-cell populations due to a failure of central and peripheral tolerance, triggering apoptosis is required for maintaining immune homeostasis (Baumgart and Carding, 2007, Ota and Das, 2015, Parigi et al., 2015).

Study by (Lee et al., 2016) approved that TNF- α mediate autoimmunity by treating mice with anti-TNF-α anti-body with a dose of LPS the result showed that Th2 cell decreased with the significant reduction in the IL-23/IL-17 level. Study by (Sartor, 2006) found that T cells in ulcerative colitis and Crohn’s disease patients are resisting apoptosis lead to over production of effector Th1 and Th2 cells, this resistance to apoptosis is enhanced by pro-inflammatory cytokine TNF-α and IL-6 while inducing apoptosis by anti-TNF-α treatment attenuate colitis.

2.4.10. Omentin activates AMP-activated protein kinase (AMPK)

The anti-inflammatory mechanism of omentin is by activation of AMPK suppressing JNK signaling pathway and reducing endothelial inflammations (Wang, 2014). Study by (Yamawaki et al., 2011) showed that omentin pretreatment (300 ng/ml) induced phosphorylation of 5' adenosine monophosphate-activated protein kinase (AMPK) through phosphorylation of threonine (Thr172) and phosphorylation of serine (Ser 1177) induced endothelial nitric oxide (NO) synthase (eNOS).

2.4.11. Omentin level association with cytokine Interleukin-6 (IL-6)

A study by Zhong et al. (2011) approved the negative association between serum omentin and IL-6 level in patients with coronary artery disease (CAD). Anti IL-6 receptor antibody induces apoptosis and attenuate ulcerative colitis (Sartor, 2006). Most of the IL-6 express in the stromal vascular fraction is positively correlated with BMI and free fatty acid levels (Hajer et al., 2008).

2.4.12. Omentin inhibits nuclear factor kappa NF-kB

Omentin inhibits NF-κB signaling pathway stimulated by proinflammatory cytokines such TNF-a and IL-1 and attenuating inflammation (Bonizzi and Karin, 2004, Wang, 2014). NF-κB stimulated by Toll-like receptor (TLR4) is essential for lymphocyte proliferation and suppressing apoptosis by expression of cyclooxygenase 2 (COX-2) (Bonizzi and Karin, 2004, Lawrence, 2009).

3. MATERIALS AND METHOD

3.1 Materials

3.1.1. List of instruments

No.	Instrument	Company	Country
1.	Centrifuge	Sigma1-14	UK
2.	Mini-centrifuge	myFUGE	Germany
3.	Polymerase chain reaction (PCR)	VWR thermal cycle	USA
4.	Nanodrop spectrometer	mySPEC	USA
5.	Vortex	Scilogex mx-s	Germany
6.	Sensitive Balance	Kern pls	Germany
7.	Gel Electrophoresis	mupid-EXU	Japan
8.	UV transilluminator (gel documenter)	Loccus	USA
9.	Microwave	Archilke	Turkey
10.	Incubator	Loccus	Germany
11.	Freezer (-20 °C)	Bosch	Turkey
12.	Refrigerator	Bosch	Turkey

3.1.2. List of tube and pipette

No.	Instrument	Sizes
1.	Micropipette	different sizes 1-1000 µL (Pipette man, Germany)
2.	Micropipette tips	different sizes 1-1000 µL
3.	Micro-centrifuge tubes	1.5 mL
4.	PCR wells	0.2 mL
5.	EDTA tube	5 mL
6.	Elution tube	1.5 mL

3.1.3. List of chemicals.

No.	Chemicals	Amount
1.	Ethanol 99%	99%
2.	water DNase, RNase free dH2O store at 20 ºC	38 µL
3.	Ethidium bromide 10 ml	2.5 µL
4.	Loading dye	1.5 µL
5.	TBE (tris-borate-EDTA) buffer	100 mL
6.	Agarose powder	2 g
7.	100 bp DNA ladder (0.5 µg / µl)	2 µL

3.1.4. Genomic DNA extraction kit content for 250 samples (Jena Bioscience, Germany)

No.	Agents	250 preps
1.	Blood Lysis Buffer	2*137 mL
2.	Lysis Buffer	80 mL
3.	Binding Buffer	80 mL
4.	RNase A (50mg/ml)	5.5 mg before use 150 µl double distilled water store at -20 º C were added
5.	Proteinase (10mg/ml)	5.5 mg before start 500 µl dd-water to the Proteinase K tube store at -20 º C were added
6.	Activation Buffer	30 ml
7.	Washing Buffer	Add 120 µl 96-99% Ethanol to each bottle (not included in the kit)
8.	Elution Buffer	25 mL
9.	Spine columns	250 pieces
10.	2 ml collection tube	250 pieces

3.1.5. Primers kit (BioLabs, New England) dilute 20 µl for 180 dH_2O

No.	primers	Sequence	Amount
1.	Forward	5′-**GAGCCTTTAGGCCATGTCTCT**-3'	1.5 µL for each PCR wells
2.	Reverse	5′-**CTCTCCTTCTTCTCCAGCCCAT**-3'	1.5 µL for each PCR wells

3.1.6. PCR-mixture (Mastermix)

No.	Master mix	For 1 reaction
1.	Taq DNA polymerase	0.5 µL
2.	dNTP mix, 2 µL each (dATP, dCTP, dTTP, dGTP) dilute 200ml +800 ml DW	3 µL
3.	Taq buffer	3 µL
4.	MgCl2 (thermostable DNA polymerase)	1.5 µL
5.	Forward primer	1.5 µL
6.	Reverse primer	1.5 µL
7.	Water nuclease free	12 µL

3.1.7. Taq polymerase kit content (Thermo scientific, USA)

No.	Materials	Amount
1.	Taq DNA polymerase	10* 500 U
2.	Taq buffer with KCl	2*1.25 mL
3.	Taq buffer	2*1.25 mL
4.	25 mM $MgCl_2$	2*1.25 mL

3.1.8. Restriction enzyme kit AccI (BioLabs, New England)

No.	Materials	Amount
1.	AccI Restriction enzyme (10U/ml)	1 µL
2.	10 X buffer	5 µL
3.	DNA product (amplicon)	6 µL
4.	Nuclease free water dH_2O	38 µL

3.2. Method

3.2.1. Subject samples

A total of 39 unrelated male and female patients, aged between 19 to 70 years old clinically diagnosed with Ulcerative colitis by specialist doctors in Onikişubat Hospital, Kahramanmaraş, Turkey from the beginning of the August 2015 to the end of the February 2016 key were included in this study. All patients were already diagnosed and they have regulatory visited Onikişubat Hospital. The diagnoses of UC were made based on usual clinical, radiological, endoscopic and histological criteria.

3.2.2. Control sample

Blood samples from 31 individuals visiting the Onikişubat Hospital, Kahramanmaraş, Turkey aged between 21 to 64 years old suffering from other diseases were collected. The participants were not fully healthy persons, and they were subjected to all tests done for the patient group.

3.2.3. Clinical diagnosis

Physicians depended on clinical signs to diagnose ulcerative colitis such as bloody diarrhea, abdominal pain, flatulence and pus in stool, also using colonoscopy as a macroscopic extent for investigating disease activity.

3.2.3.1. Measurements

In addition to the age, gender, body height, and body weight of the subjects, the history of patients, disease stages (active or remission), disease duration, disease starting age, patient habits (cigarette, maraş otu, alcohol) and current therapies used by UC patients were recorded in detail.

3.2.3.2. Anthropometric Measurements

Body mass index (BMI) was calculated as weight in kilograms divided by height squared in meters (kg/m^2) according to the criteria of the World Health Organization.

3.2.3.3. Laboratory investigation

The laboratory analysis such as Complete Blood Count (CBC), hemoglobin (Hb), Hematocrit (Ht), White Blood Cell Counts (WBC) and Platelet Count (PLT) were measured.

3.2.3.4. Biochemical tests

Fasting Blood Glucose, Blood Urea Nitrogen (BUN), Creatinine, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), Gamma-glutamyl Transpeptidase (GGT), Total Bilirubin (T.Bil), Direct Bilirubi (D.Bil) were evaluated. Clinical laboratory indexes included Erythrocyte Sedimentation Rate (ESR), C-reactive protein (CRP), were also measured to estimate the severity of ulcerative colitis.

3.2.4. Blood collection

5 mL of venous blood was obtained from the patient and control groups and collected in EDTA tubes for extracting genomic DNA from peripheral blood leukocytes. They were transported within a cool box to the genomic laboratory in Sütçü imam University in Kahramanmaraş, and then the samples were stored at 4 °C until analysis.

3.2.5. Extraction of genomic DNA from blood

The spin column based nucleic acid extraction kit (Jena Bioscience, Germany) was used for isolation of genomic DNA from whole blood. This method completely removed PCR inhibitors such as proteins. The obtained DNA was suitable for a variety of applications, including real-time PCR, southern blot analysis, genotyping and validation of the SNP.

3.2.5.1. Sample preparation

A volume of 200 µL of the blood was mixed with 1000 µL of blood lysis buffer in 2 mL micro-centrifuge tube (for lysing RBC) and incubated for 10 min on ice with briefly vortexing 2-3 times. Then, the tube was centrifuged at 10.000 rpm for 10 min to pellet the white blood cells. Later, the supernatant was completely discarded and the leukocyte pellet at the bottom of the tube was kept.

3.2.5.2. Cell Lysis

300 µL of the lysing buffer and 2 µL of RNase were added to the cell pellet with vortexing vigorously for 30-60 sec. Then, 8 µL of the proteinase K was added and mixed by pipetting. Next, the mixture was incubated at 60 º C for 10 min and cooled down for 5 min. Later, 300 µL of the binding buffer was added with vortexing briefly. The tubes were put on ice for 5 min and centrifuged for 5 min at 10.000 rpm.

3.2.5.3. Column Activation

A spin column was placed into the 2 mL collection tubes and 100 µL of activation buffer was added to the spin column. The tubes were centrifuged at 10.000 rpm for 30 sec. and the flow-through was discarded.

3.2.5.4. Column loading

The supernatant was transferred by pipette directly to the spin column and centrifuged for 1 min at 10.000 rpm, and then the flow-through was discarded.

3.2.5.5. Primary washing

500µL of the washing buffer was added to the spin column and centrifuged for 30 sec. at 10.000 rpm, and then the flow-through was discarded.

3.2.5.6. Secondary Washing

500 µL of the secondary washing buffer was added to the spin column and centrifuged for 30 sec. at 10.000 rpm. The flow-through was discarded. The spin column was centrifuged at 10.000 rpm again for 2 min, and the flow-through was discarded. The 2 mL micro-centrifuge tube was discarded and the spin column placed into a new the collecting tube.

3.2.5.7. DNA Elution

50µL of the elution buffer **(**dH2O pre-heated at 60 ºC) was added to the center of the column, then the samples were incubated at room temperature for 1 min and centrifuged at 10000 rpm for 2 min. The eluted DNA stored at 4 º C or at-20 º C.

3.2.6. Measuring DNA concentration by NanoDrop Spectrometer

The NanoDrop (mySPEC, USA) was used to quantify and assess the purity of DNA, average DNA concentration of 70 samples (39 patients and 31 controls) were 19.1 ng/µL.

The sample reader of the NanoDrop was washed by distilled water and dried with a KimWipe then 2.5 µL of the elution water (blank) loaded and blank button clicked for resetting the software, later 2.5 µL of the DNA sample loaded and changing the computers setting to DNA then the measure button clicked and the amount of DNA measured by (ng/µL) then the sample reader cleaned again.

3.2.7. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay.

The genotyping of omentin gene Val109Asp SNP was determined by Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay.

3.2.8. Polymerase chain reaction (PCR) for DNA amplification

Omentin gene consists of 12.233 bp with 8 exons and 7 introns, the 471 bp of DNA fragment of omentin sequence containing Val109Asp SNPs in the exon 4 were amplified by PCR (Thermal cycler VWR, USA), for this purpose the specific oligonucleotide primers (BioLabs, New England) forward primer; 5′-**GAGCCTTTAGGCCATGTCTCT**-3′, and reverse primer; 5′-**CTCTCCTTCTTCTCCAGCCCAT**-3′ were used, the A/T single nucleotide polymorphism and 471 bp of omentin fragment show in Figure3.2.1

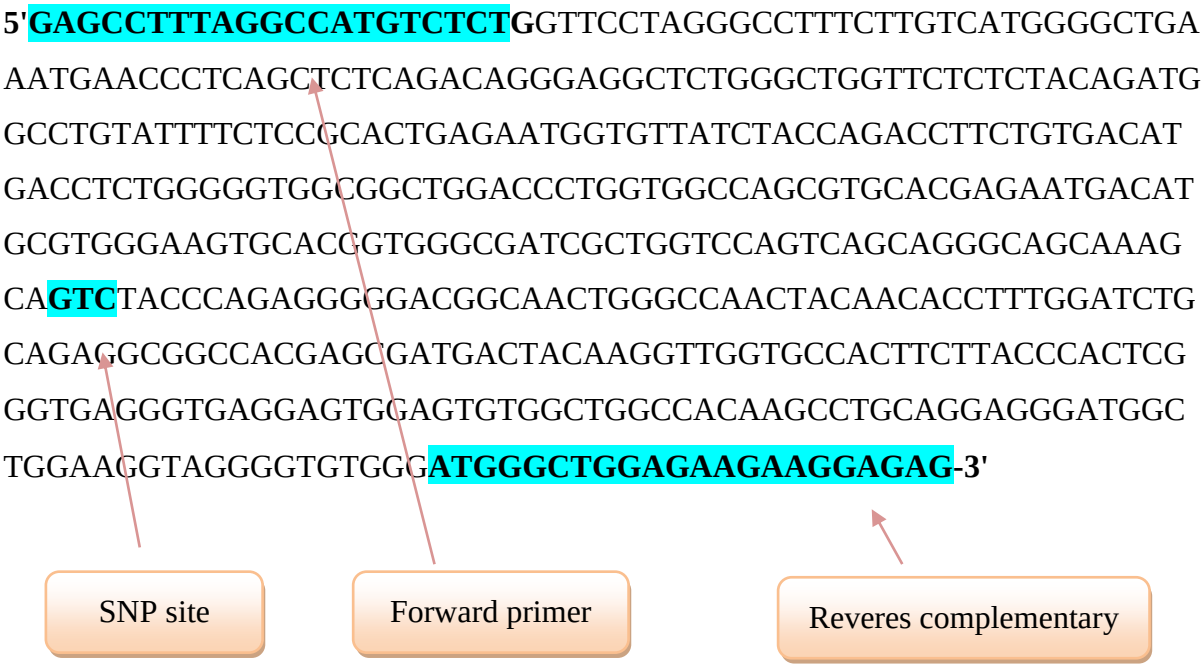


Figure3.2.1. amplifying 471 bp of omentin DNA sequence by PCR with forward and reveres complementary sequence and A/T site of SNP

3.2.9. Preparing PCR master mix

PCR master mix was prepared to minimize the possibility of pipetting errors, all the solutions were vortexed gently and centrifuged briefly. After thawing, the PCR master mix was prepared for amplifications of 70 samples (39 patients and 31 controls) by mixing 0.5 µL of Taq polymerase from (Thermo scientific, USA) kit, 3 µL of dNTP, 1.5 µL of forward and reverse primers, 12 µL of water nuclease free (dH2O), 1.5 µL of $MgCl_2$, and 3 µL of Taq buffer.

23 µL of the PCR mixture was placed into a thin-wall PCR tube for each reaction, then 2µL of the DNA template added, the loaded PCR thin-wall put in mini-centrifuge for 30 sec.

The DNA of omentin gene wasamplified by PCR cycling performed under the following conditions: pre- denaturation at 96°C for 5 min., followed by 36 cycles each cycle was optimized for denaturation template at 94°C for 30 sec., annealing of the primers to the template at 58 °C for 30 sec., and extension at 72°C for 40 sec. while the final extension temperature was at 72°C for 7 min.

3.2.10. Electrophoresis

Electrophoresis (mupid-EXU, Japan) used for separating PCR products (amplicon).

3.2.11. Preparing agarose gel

2g of the agarose powder put in 100mL TBE (Tris/Borate/EDTA) buffer on a beaker, the mixture was put in the microwave at 100°C until the agarose is dissolved, and then the gel was put in a room temperature for cooling down to 65 ºC and 2.5 µL of ethidium bromide added to agarose gel. The comb was put on the electrophoresis tray then sufficient amount of agarose gel was poured into the tray, and pleased for about 30 minutes to become solid. The TBE buffer was put in the buffer chamber, once the agar has solidified. The comb was removed and the gel was placed in the chamber.

3.2.12. Loading gel

1.5 µL of the loading dye was mixed with 7 µL of the amplicon by pipetting. The mixture was loaded into the gel wells carefully. 2 µl of the DNA ladder 100 bp from (Thermo Scientific, USA) kit and 1.5 µL of loading dye were mixed and loaded in the last gel well. Once all the samples were loaded, the gel box was attached to the power supply and run at 135V for 30 minutes Figure3.2.2.

Figure3.2.2. The gel electrophoresis after 24 min of running 471 bp of amplified DNA of omentin gene.

3.2.13. UV Transilluminator for Gel documentation (Loccus, USA)

After electrophoresis was run for amplicons, computerized gel documentation was used for visualizing and analyzing DNA bands. The gel showed one band with 471 bp of omentin gene under the UV light Figure3.23.

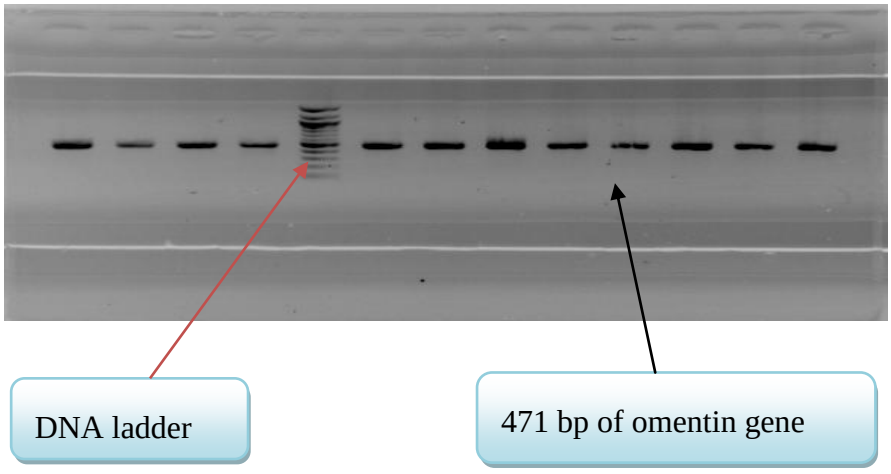
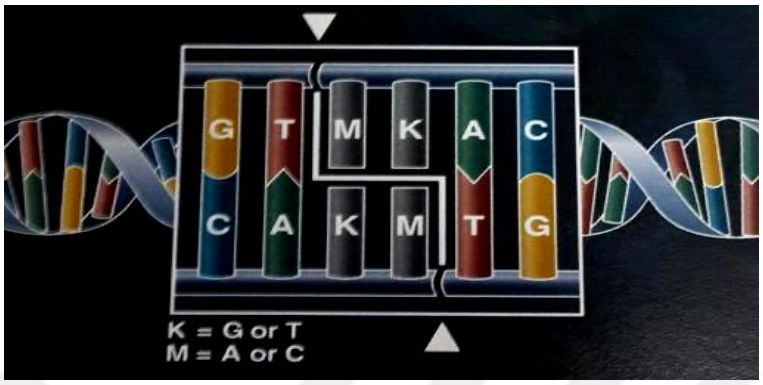


Figure3.2.3 UV showing omentin gene amplicon with 471bp band after running by gel electrophoresis

3.2.14. Restriction enzyme fragment length polymorphism (RFLP)

Omentin gene amplicon were digested with the AccI restriction enzyme (BioLabs, New England) kit by mixing Accl l µL, PCR product 6 µL, 10X buffer 5 µL and dH_2O 38 µL then incubated at 37C° overnight. The AccI cut site shown in figure3.2.4.



Enzyme	DNA sequence	Cutting site
AccI	5' GTMKAC	5' ---GT MKAC--- 3'
	3' CAKMTG	3' ---CAKM TG--- 5'

Figure3.2.4. Restriction enzyme AccI cutting site digest the 471 bp of omentin gene with Val109Asp genotype

The digested products of omentin gene by AccI enzyme were separated by 2% agarose gel stained with 2.5µl ethidium bromide and the gel was run at 135V for 30 minutes Figure3.2.5. Agarose gel of DNA fragment bands was documented by UV light.

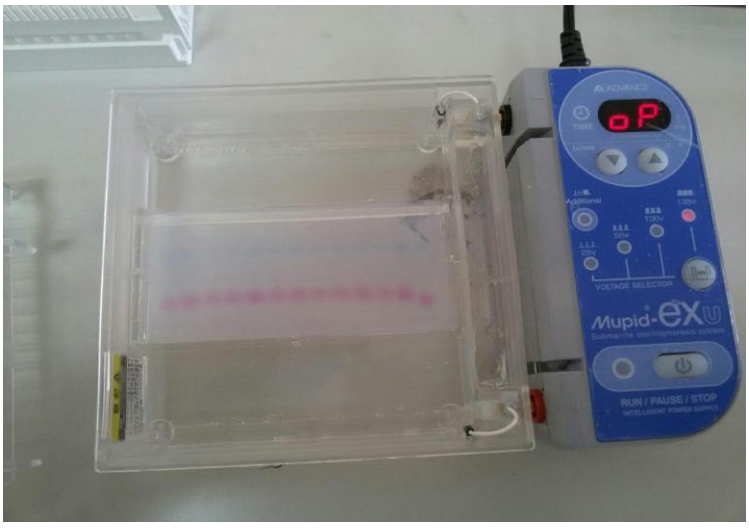


Figure3.2.5. Running DNA fragments of omentin in the gel electrophoresis after cutting by AccI enzyme.

After digestion Omentin-1 gene with Asp/Asp (GAC/GAC) homozygotes genotype has no digestion sites show single band (471 bp). While amplified Omentin-1 rs2274907 SNPs with val109Asp cut by the Accl enzyme in the presence of the T allele, the Val/Val (GTC/GTC) homozygotes genotype cut into two fragments showing two bands with 274bp and 197 bp sizes and Val/Asp (GTC/GAC) heterozygotes genotype generates three bands with (471bp, 274bp and 197bp) sizes, the fragments length after digestion are illustrated in (Fiqure3.2.6)

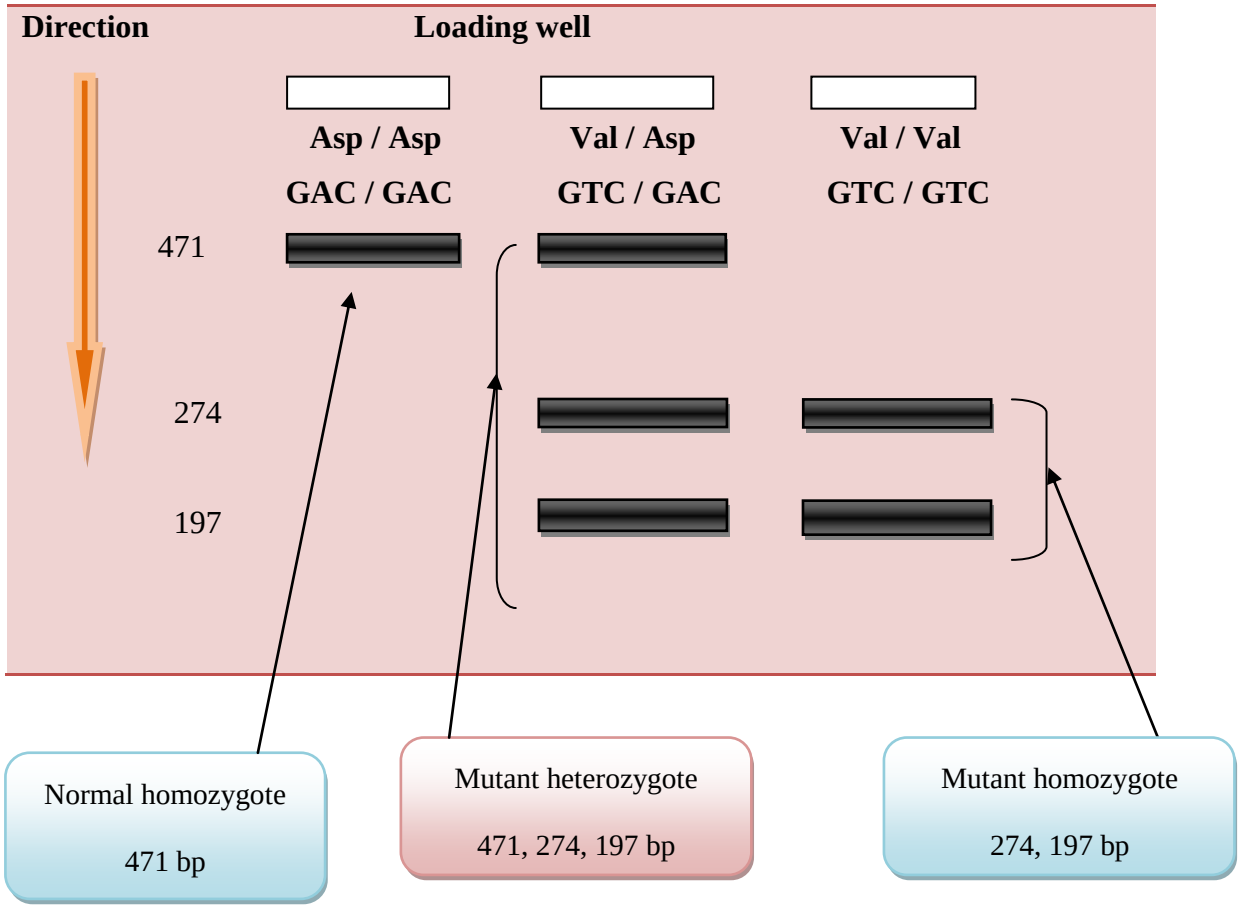


Figure3.2.6.RFLP method for cutting 471 bp of omentin gen, the Val109/Asp polymorphism cut by restriction enzyme producing Asp/Asp (GAC/GAC) homozygote one band, Val/Val (GTC/GTC) homozygote mutant tow bands Val/Asp (GTC/GAC) heterozygote mutant three bands.

3.3 Statistical analyses

Statistical Package for Social Sciences software (SPSS 17, Chicago, IL, USA) was used for analysis Descriptive parameters were shown as means± standard division and percentages, also Odds ratios and their confidence intervals based on genotype frequency were measured by SPSS. p value was measured and the values of $p<0.05$ were considered as a statistically significant. Chi-square tests were used to determine whether a significant relation existed between the variables, and when the p value was found to be less than 0.05, it was considered as the evidence of a correlation between them. Abnormally distributed data among genotype groups compared by means± standard

4. RESULTS AND DISCUSSION

4.1. Results

Association of the genetic variation of omentin gene Val109/Asp single nucleotide polymorphism (SNP) was assessed in our study between patients suffering from ulcerative colitis comparison with the control group. For our purpose, a total of 70 subjects were enrolled in this study, consisting of 39 patients cases (23 men and 16 women with a mean age of 39.30 ± 15.25) diagnosed with ulcerative colitis by usual clinical, radiological, endoscopic and histological screening also detecting disease stages and 31 individuals (10 men and 21 women with a mean age of age 41 ± 13.71) suffering from other disease, who were served as the control group.

4.1.1. Gender

There was no difference between patients gender, they were (41.02%) female and 58.97% male this indicating that both gender are susceptible for Ulcerative colitis.

4.1.2. Disease stage

Not all patients group were at active stage, 35.89% were at remission stage, while 64.1% of our patient were at active stage.

4.1.3 Anthropometric results

Our results show that there was no significantly difference in age between groups. Also there were no significant difference in BMI, however, the mean result show decreased BMI in Val/Asp it was 20.02 while increased in Asp/Asp it was 26.05 (Table 4.1.1). However the mean BMI of patients during remission stage was 25.68 while mean result of BMI during sever stage was 23.23. Decreasing of BMI according to Val/Val genotype is showing in Figure4.1.1.

4.1.4 Patients habits

Of all patients, 89.7% were non-smokers, and 87.17% were non-alcoholic and 82% were non- maraş out smoker.

Parameters	Asp/Asp	Val/Asp	Val/Val	P value
	n=33	n= 28	n= 9	
Age (year)	39.93 ± 14.25	47.15 ± 13.38	48.28 ± 18.83	0.24
Weight (kg)	69 ± 10.68	58 ± 15.27	62.33 ± 7.31	0.12
Height (cm)	165.35 ± 9.09	162.87 ± 7.49	165.2 ± 3.27	0.77
BMI kg/m^2	26.05 ± 12.82	20.02 ± 12.32	22.89 ± 9.86	0.39

Table4.1.1. Anthropometric measurement for controls and patients groups according to the genotypes.

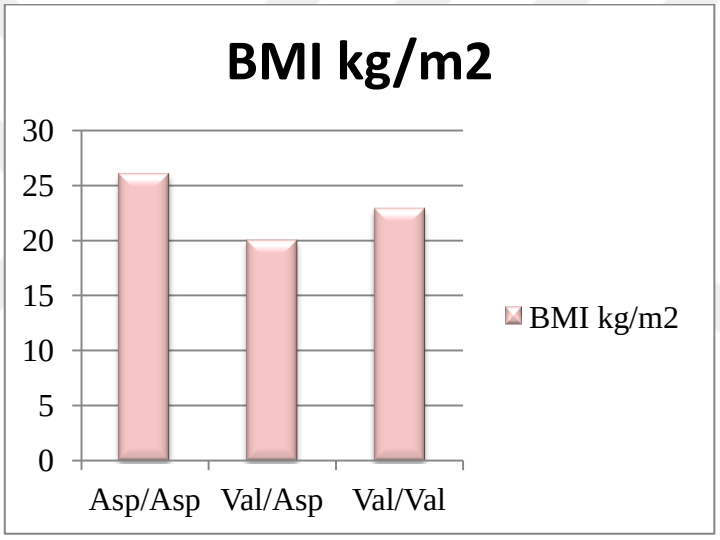


Figure4.1.1. Decreasing of BMI according to Val/Asp genotype compared with Asp/Asp

4.1.5. Laboratory investigation

In our study hemoglobin (HB) and hematocrit (Hct) levels were significantly lower in patient group compared to the control group and p value were ($p<0.05$), A complete blood count is done to check for anemia and thrombocytosis, HB and Hct decreasing in UC patients indicating severity of the disease and they suffer from anemia because of bloody diarrhea. However there were no significant difference according to the WBC and PLT the mean result show that WBC and PLT increase in UC patients because of over production T-cells in response to inflammation in UC Table4.1.2.

Parameters	UC (39)			Control (31)			P value
HB g/dl	11.92	±	2.31	13.31	±	1.56	0.05
Hct %	36.65	±	6.96	40.15	±	4.42	0.016
WBC (cm)	8.99	±	3.54	8.54	±	2.08	0.911
PLT	315.43	±	105.13	272.22	±	63.86	0.149

Table4.1.2 HB and Hct levels were significantly lower in patient group compared to the control group and Pvalue were ($p<0.05$) indicating anemia, increasing inflammatory marker WBC and PLT indicate severity of UC.

4.1.6 Laboratory investigation according to genotype

Result of our study show that there were no significant correlation between genotypes according to the HB, Hct, WBC, and PLT, however the mean result show that omentin SNPs may positively correlated with severity of the UC by decreasing HB and Hct in Val/Val genotype mean of HB was (11.65) and Hct was (35.84), also increasing mean of WBC and PLT in Val/Val genotype show that omentin may associated with inflammation Table4.1.3. Decreasing HB and increasing WBC in Val/Val genotype showing in Figure4.1.2. Also omentin SNPs increasing thrombocytosis in Val/Val showing in Figure4.1.3.

Parameters	Asp/Asp n=33			Val/Asp n= 28			Val/Val n= 9			P value
HB g/dl	12.71	±	2.44	12.47	±	1.82	11.65	±	1.82	0.51
Hct %	38.83	±	7.31	37.84	±	5.10	35.84	±	5.15	0.51
WBC (cm)	8.26	±	1.97	8.76	±	2.63	11.22	±	6.12	0.07
PLT	284.62	±	83.09	293.42	±	70.92	317.66	±	109.4	0.33

Table 4.1.3 Results of laboratory testes HB, Hct, WBC, PLT according to genotypes

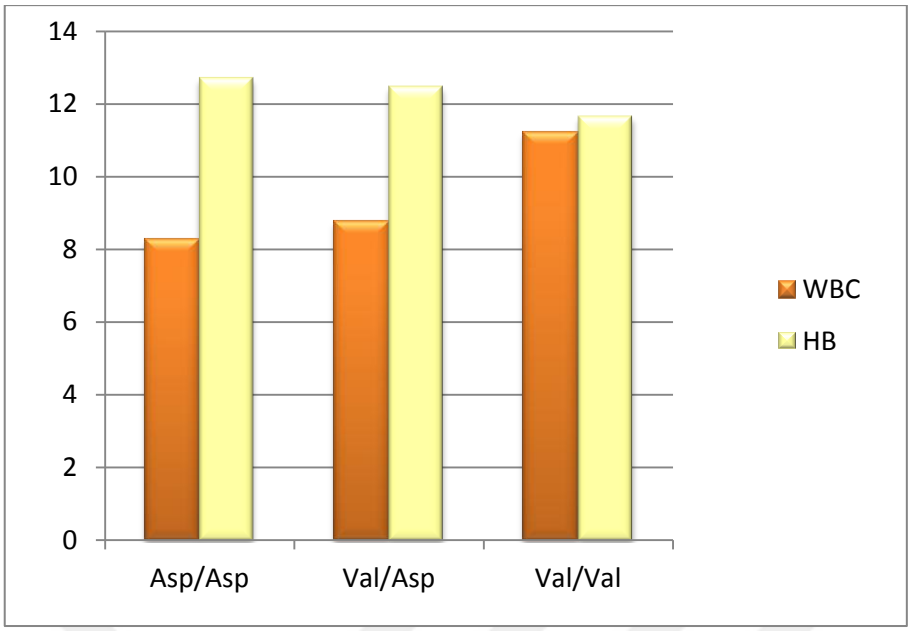


Figure 4.1.2 Increasing WBC and decreasing HB in Val/Val genotype

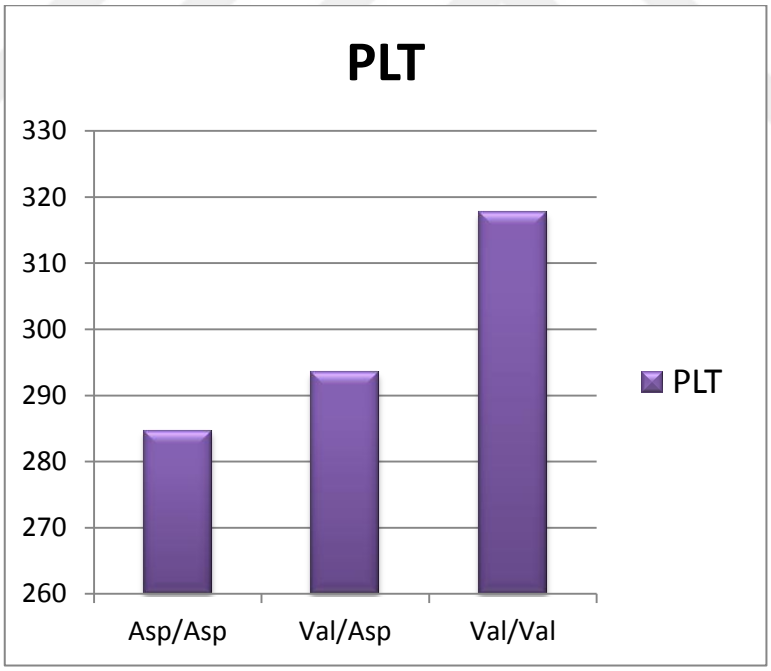


Figure 4.1.3 Increasing inflammatory marker PLT in Val/Val genotype.

4.1.7. Biochemical tests

There were no significant difference of inflammatory biomarkers between patients and control group, however mean result showed that BUN as a kidney inflammatory marker increased in UC group, while mean result of ALP, Gamma-glutamyl transpeptidase (GGT), total bilirubin (T.Bil), Direct bilirubin (D.Bil), Erythrocyte Sedimentation Rate ESR, and C-reactive protein CRP as a liver inflammatory biomarker increased in UC group compare with control group Table4.1.4.

Parameters	UC (39)			Control (31)			P value
Glucose (mg/dL)	92.48	±	29.97	93.1	±	19.38	0.386
BUN (mg/dL)	15.03	±	5.20	11.94	±	3.05	0.128
Cre (mg/dl)	0.67	±	0.25	0.71	±	0.15	0.89
AST (U/L)	22.00	±	8.39	23.45	±	6.23	0.221
ALT (U/L)	21.91	±	9.81	25.83	±	12.04	0.253
ALP (U/L)	110.83	±	114.30	68.75	±	28.22	0.948
GGT (U/L)	45.80	±	61.86	23.11	±	14.89	0.567
T.Bil (mg/dL)	0.99	±	0.82	0.58	±	0.18	0.363
D.Bil (mg/dL)	0.38	±	0.49	0.2	±	0.09	0.789
ESR (mm/hour)	29.43	±	19.13	24.75	±	18.14	0.688
CRP (mg/L)	22.22	±	43.01	5.57	±	2.82	0.676

Table4.1.4 Comparison of clinical and demographic characteristics of the study groups variables are represented as mean ± SD and P values.

4.1.8. Biochemical test according to genotypes

The inflammatory biomarker were further analyzed according to the genotype groups, there were significant difference between genotypes in T.Bil (0.008), D.Bil (0.0001) and GGT according to Val/Val genotype indicating severity of the disease Figure 4.1.4, while other inflammatory biomarker such alkaline phosphatase (ALP), ESR, and C-reactive protein CRP show no significant difference, however mean result show that Val/Val genotype may associated with severity of inflammation, while mean of glucose decreased in Val/Val genotype indicating malabsorption in UC patients Table 4.1.5.

Parameters	**Asp/Asp n=33**			**Val/Asp n= 28**			**Val/Val n= 9**			**P value**
Glucose (mg/dL)	95.41	±	34.46	93.36	±	16.35	79.66	±	8.01	0.42
BUN (mg/dL)	16.21	±	5.29	12.59	±	3.90	11.00	±	2.00	0.12
Cre (mg/dl)	0.70	±	0.22	0.66	±	0.22	0.60	±	0.07	0.49
AST (U/L)	23.27	±	6.69	21.57	±	5.61	24.40	±	15.04	0.669
ALT (U/L)	23.85	±	10.71	23.66	±	11.65	21.57	±	9.74	0.883
ALP (U/L)	56.66	±	18.14	72.22	±	26.00	197.50	±	204.35	0.073
GGT (U/L)	29.33	±	21.53	24.63	±	18.02	109.50	±	143.54	**0.044 ***
T. Bil (mg/dL)	0.59	±	0.29	0.73	±	0.70	1.85	±	0.18	**0.008 ***
D.Bil (mg/dL)	0.17	±	0.07	0.17	±	0.09	1.14	±	0.50	**0.0001 ***
ESR (mm/hour)	9.32	±	7.13	25.46	±	13.95	31.25	±	20.27	0.656
CRP (mg/L)	25.44	±	51.26	6.59	±	5.94	39.43	±	48.27	0.197

Table4.1.5. Comparison of clinical characteristics according to the genotypes. Data are represented as mean ± SD. Categorical variables are displayed as number of patients.

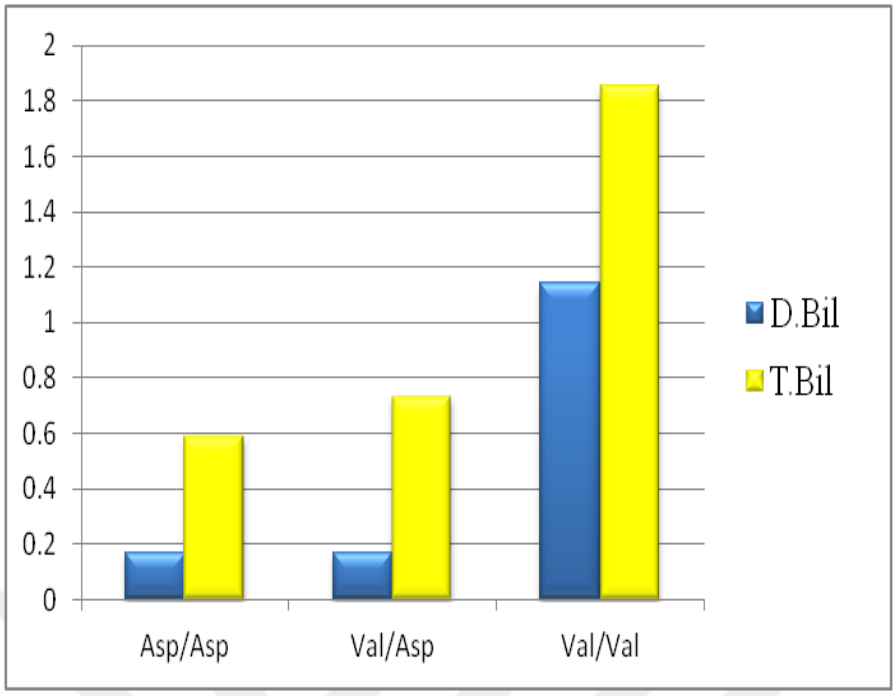


Figure4.1.4 Significant differences in T.Bil (0.008) and D.Bil (0.0001) according to Val/Val genotype indicating omentin polymorphism correlated with the severity of the disease.

4.1.9. Omentin genotypes Val109Asp polymorphism

The genotypes of omentin gene Val109Asp SNP was determined by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. 471 bp of omentin gene was amplified and AccI restriction enzyme used to cut in present of Val109Asp SNP, after cutting the fragment bands documented by UV electrophoresis the Asp/Asp (GAC/GAC) homozygote with no cutting site showed one bands 471 bp, while Val/Val (GTC/GTC) homozygote mutant cut by the restriction enzyme producing two bands (274, 197)bp and Val/Asp (GTC/GAC) heterozygote mutant cut by the AccI enzyme showing three bands Figure4.1.5.

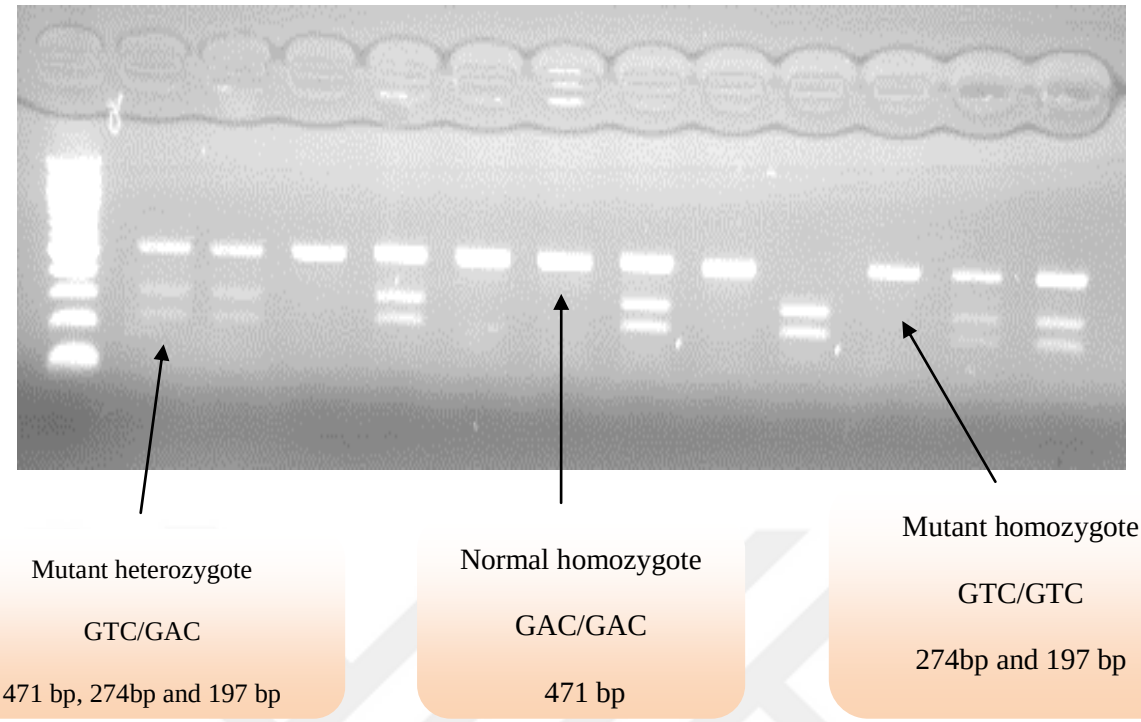


Figure4.1.5. UV electrophoresis show RFLP assay digest GTC site by AccI restriction enzyme produce different fragment of omentin gene of patients sample

The differences in genotypes between patients and control measured according to the band showed by UV light, the patient's group genotypes were Asp/Asp (48.7%) 19 patients, Val/Asp genotype were 14 patients (35.9%), and Val/Val 6 patients (15.4%) while in the control group genotypes were Asp/Asp 14 individuals (45.15%), Val/Asp 14 individuals (45.15%) and Val/Val 3 individuals (9.7%) of the cases, Omentin Val109Asp genotypes distributions, P value, the ORs and Cl for each genotype were given in the Table 4.1.6.

Genotype	Total n=70 (%)	UC n=39 (%)	Control n=31 (%)	P value	Odd ratio
Normal Homozygote % (GAC/GAC)	33 (47.1 %)	19 (48.7 %)	14 (45.2 %)		0.368 (0.13 to 0.99)
Heterozygote % (GTC/GAC)	28 (40.0 %)	14 (35.9 %)	14 (45.1 %)	**0.652**	1.31 (0.5 to 3.4)
Mutant Homozygote % (GTC/GTC)	9 (12.9 %)	6 (15.4 %)	3 (9.7 %)		4.35 (0.86 to 21.87)

Table4.1.6 Comparison of the Omentin Val109Asp SNP genotypes distributions in UC patients and control, data are represented as percentages, genotype frequencies are measured by P value using chi-square and odd ratio (OR), confidence interval (Cl), (Val/Val=GTC/GTC, Val/Asp=GTC/GAC, Asp/Asp=GAC/GAC) .

In our data, we could not find significant difference between ulcerative colitis and control subjects patients regarding to omentin gene SNPs Val109Asp polymorphism the Pvalue result was P>0.05, however after measuring the odds ratios and their confidence intervals based on Asp/Val distribution, genotype of Val/Val mutant homozygous was 4.35 heterozygous Val/Asp genotype was 1.31 while the OR for normal homozygote Asp/Asp was 0.368, this result showing Val/Val genotype may slightly associated with the ulcerative colitis that may increases the incidence of ulcerative colitis. Genotypes difference between patient and control showing in Figure4.1.6

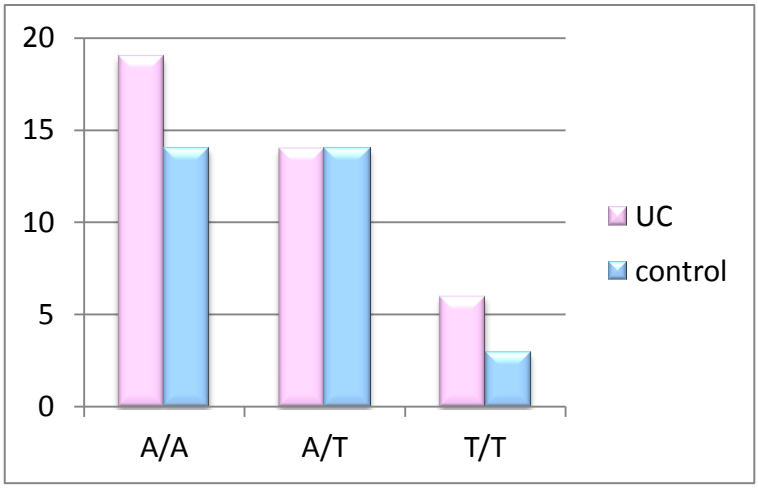


Figure 4.1.6 Genotypes between Ulcerative Colitis patients and control groups showing the increasing of Val/Val genotype in patients group.

4.2. Discussion

To our knowledge, our research is the first study searched the association between omentin gene Val109Asp polymorphism and ulcerative colitis. The result of our study showed that there was no significant difference regarding to the Val109Asp polymorphism genotypes between the control and patient groups (P>0.05). This result may be due to the fact that our control group did not consist of completely people, but, instead, of people who visited the Hospital with some health complaints. These people may be suffering from other disease such as Crohn's disease which is the subcategory of inflammatory bowel disease or they may have metabolic syndrome such as rheumatoid arthritis, diabetes, cardiovascular disease and the other autoimmune diseases that may contribute to the UC. The correlation between the genotypes and the health status of the people might have been found as convincing if a better control group, in which only people with no autoimmune disease are involved, could be used.

On the other hand, our study showed that the omentin Val109Asp SNPs may be slightly correlated to the ulcerative colitis because the homozygous mutant genotype Val/Val was found 4 fold more in patients with UC than the control group the Val/Val was (OR=4.35). These circumstances might clarify why the result wasn't significant and the p-value was p>0.05. Therefore, the results of this study should be confirmed by further studies perform with larger patient groups with more appropriate subjects to clarify this issue and assessing omentin plasma levels according to genotypes.

Our result was the same as the result of a previous study conducted by Yörük et al., (2014). In this study, the researchers found, similarly to what we observed, that there was no significant difference between genotypes of omentin Val109Asp polymorphism with coronary artery disease while the odd ratio result of Val/Val genotype was 3.46. Also, the result of study by Yaykasli et al., (2013) was revealed as similar to our results, declaring no significant difference between Rheumatoid arthritis patient and control group according to the Val109Asp polymorphism.

Our data cannot provide certainty that cytokine omentin is associated with UC. The similarity in genotype of theVal109Asp SNPs between healthy controls and UC patients indicate that the amino acid at the position 109 could not be important for the cytokine omentin signaling, May be the Val109Asp is not more than a single nucleotide polymorphism (SNP) and does not cause actual disease. The exact function of omentin in

human physiology and omentin specific receptor is unknown so far. Certain factors can control pre/post-transcription of omentin gene expression, thereby, Omentin sequence variations, mRNA expression levels, receptor, interaction between different pathways and the specific function of omentin in the ulcerative colitis emphasize this study further.

Human omentin protein have two N-glycosylation sites at amino acid Asn 154 and at Asn163 but not at the 109 Asp, the Asp109 (Aspartic acid) may not represent conserved amino acid, (Schäffler et al., 2007). Maybe the amino acid at the position 109 could not be important for the omentin protein function, this make reasonable why the frequency of the Val109Asp does not significantly differ between UC patients and controls group.

Study by Yin et al. (2015) proved that omentin-1 plasma levels significantly lower in active UC and CD, indicated that serum omentin-1 levels could be estimated as a biomarker of the presence and severity of UC. Under these debates, our studies carried out in order to prove the anti-inflammatory effect of omentin in ulcerative colitis. As a result of our study, it was observed that the inflammatory biomarker T.Bil and D. Bil significantly increased in Val/Val genotype, which approved the study mentioned above. Additionally, the mean results of Hb and Hct in Asp/Asp genotype in our study may indicate that omentin prevent anemia by improving UC.

The mean result of BMI in our study show that our patients were among lean persons, and this was contrast of other study demonstrating that inflammation positivity related with BMI. One of such studies was conducted by Turan et al. in 2014. In their investigation, the mean value of BMI increased in patients with psoriasis who also carried Val109Asp polymorphism. According to their results, the author suggested that the metabolic syndromes may more likely to occur among obese individuals. However, because of the fact that our patient suffered from bloody diarrhea, malnutrition, loss of appetite, they are prone to lose weight thereby they commonly have decreased BMI. Mean BMI of our patients during active stage was 23.23 while our patient in remission stage was 25.68, which also clearly indicate the relationship between the severity of the disease and the reduction in BMI.

Decreased levels of omentin have been suggested to be a risk marker for metabolic syndromes such as Crohn’s disease, coronary artery disease, rheumatoid arthritis, atherosclerosis, and asthma. Increase in omentin plasma levels may be a positive marker for the lean state due to omentin’s roles in energy expenditure and glucose metabolism

through activating Akt signaling pathways and endothelial nitric oxide synthase. Due to these connections, in 2004, Lee et al. actually suggested the use of omentin as a novel therapy for pathogenesis related to obesity, diabetes and inflammation. Study by Tan et al. (2010) found that omentin plasma levels were negatively correlated with obesity, body mass index (BMI) and glucose in overweight polycystic ovary syndrome PCOS patients. They also proved that serum omentin-1 increased after treatment with metformin in PCOS women, which is a treatment normally used for diabetes to enhance the insulin receptor (IR) and suppress glucose production in the liver. It has been demonstrated that excessive glucose may suppress omentin mRNA expression, pointing that omentin expression could be under control of epigenetic factors along with genotype. However, our result showed that sugar levels were decreased in the individuals with Val/Val genotype and in patients compared to the control. These findings were in contrast with other studies mentioned and it may be due to the fact that our patients suffered from lose appetite and malnutrition.

We rationalized our study by depending on several studies which characterized omentin as anti-inflammatory cytokine and the relationship of omentin with inflammation which has been reported in many studies. Del Prete et al. (2014) proved that the levels of omentin mediated inflammation were significantly reduced in the synovial fluid of patients with Rheumatoid Arthritis. Omentin eliminated inflammation by suppressing TNF-α inducing cyclooxygenase (COX)-2 expression in vascular smooth muscle cells and endothelial cells (Hamnvik et al., 2015). The protective role of omentin as anti inflammatory cytokine showed by Wang et al. (2014) by increasing inflammatory biomarker BUN, Cre, AST, ALT, CRP in coronary heart disease, these inflammatory biomarker inversely correlated with Omentin plasma levels. Considering our results, we may also hypothesis that omentin might have an anti-inflammatory effect by increasing the mean of inflammatory biomarkers (ASP, GGT, ALP, ESR, and CRP) in Val/Val genotype. To confirm our results, further studies are required using colonic biopsy sample during abdominal surgery (laparotomy) for detecting Val109Asp polymorphism by extracting DNA from colonic tissue.

Study by Silva et al. (2010) approved that infliximab (anti TNF-α antibody) drug cause apoptosis in activated T-cells but not naive T lymphocytes and increase Tregs cells levels in IBD patients, thereby causing macrophages reduction at the site of the inflammation due to inhibition proinflammatory cytokines such as IL-1 and IL‑6. Deficiency in apoptosis frequency is the cause of chronic inflammation. Omentin has anti

TNF-α role and enhance apoptosis. Pre-treatment of rat cells with Omentin in adipose tissue showed significantly decreasing of TNF-α through phosphorylation of p38 and JNK (Lapointe et al., 2014). In the light of these previous findings, along with the careful evaluation of our data, we can speculate that omentin may have apoptotic effects because of which WBC levels are measured lower in Asp/Asp genotype.

Study by Tan et al. (2010) demonstrated that adding omentin-1 (200 ng/ml) to serum significantly decreased CRP plasma levels. Our results may appear to be in accordance with the previous study since we also observed low CRP levels in the patients with Asp/Asp genotype, which produces functional omentins. However, we also need to consider that 35.89% of our patients were at remission stage, where you normally expect lower CRP levels.

Study by Regeling et al. (2016) showed that cigarette smoke suppresses inflammation by inducing T-cell apoptosis indicating that smoke has protective role on inflammation. Also study by (Shibata et al., 2011) indicated the protective role of smoking in cardiovascular disease by attenuating inflammation when the mean result of smoking was 30.7 in coronary artery patients while the mean result of non-smoker increased in control groups and it was 42.6. Our study results seem to approve this previous study because 89.7% of our patients were non-smokers.

4.2.1. Study limitations

The present study has several limitations. In our study, we used patients' blood sample for detecting polymorphism while taking biopsy from the most severely affected areas from in intestinal mucosa and sub mucosa during colonoscopy or obtaining surgical specimens of omental adipose tissue during laparotomy would give a better result. Investigation of the presence of tissue-specific Val109/Asp SNP and detecting polymorphism locally are surely needed to come to a solid conclusion about the possible association between the genotypes and the health status.

Another important limitation of our study was the small number of population. Further research with a larger study population is required.

Also omentin plasma levels were not detected for comparison with genotypes, which made our conclusions partially weak. To make strong connection between our observations and the omentin's putative role on that observation, we recommend researchers to measure plasma omentin levels of the individuals they work with.

Lastly, the control group we used in the study did not include only fully healthy individuals. There is a slight chance that some of these people carried Crohn's disease which has the same clinical complication. Besides, we did not exclude the people with metabolic syndromes and other autoimmune diseases. Because of the un-certainty of the real health status of our control group, our conclusions remained somehow controversial. Therefore, for the further studies, we strongly recommend that every participant of a similar study would be carefully examined against any inflammation related disease.

5. CONCLUSION

We can conclude from our data that there was no significant difference between control subjects and ulcerative colitis patients regarding to omentin Val109Asp polymorphism.

However, we can speculate that the Val/Val genotype may increases the incidence of ulcerative colitis because OR of TT genotype was 4.35 higher in patients compared to control group.

Omentin may play an anti inflammatory role by increasing T. Bil, D. Bil., CRP, GGT, ESR, ALP and PLT plasma levels.

Immunosuppression effect of omentin, observed as decreased WBC levels in Asp/asp genotype, in autoimmune disease may also be concluded from our data.

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APPENDIX

Omentin gene from 5` to 3` contain (12,233) base pairs with Val109/Aap SNPs (A/T) at exon 4.

5′AACGACCAAGAATAACCAAAAAAGAAGAGCAAAGTGAGAAGCCTTGCTCTACTGGATATCAAGACATTTTATCAA
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AATATTCAGAAAAGTTGAAAGGAAGTCAAGACAATAATGTCACTAAAGCTAAAGGAAGAGAGTTTTTTTTTTTTTCT
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CAGGTTCAAGCAATTCTCCTGCCTCAGCCTCCTGAGTAGTGGGACTACAGGTGCTTGCCACCATACCTGGCTAATTT
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TGGGCTCCCATCTGCAGGTGCTCATTCAGAAGGAGACATGTGGCCAAATCATCCCCTGTGGGTTATTGGTACAGATA
GGATGGGGATGGCATCAGTTCTTACCCGTGACTAAATATTAGGCATAATATTGTGTTTCCTCTACAATG-3`

PRIMERS

FORWARD	**REVERS**	**PCR**
5′-**GAGCCTTTAGGCCATGTCTCT**-3′	5′-**CTCTCCTTCTTCTCCAGCCCAT**-3′	471 bp

5′-**atggcgtggagaagaaggagag**-3′

(Reverse complementary)

CURRICULUM VITAE

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Education

Degree	Subject	Department/ University	Date of Graduate
B.Sc.	Science	College of science /biology Salahaddin University, Iraq	1995-1996

Foreign Language

English language skills, getting Score 5 in IETLS

Congress

Participating in the 3rd International Molecular Immunology & Immunogenetics Congress (MIMIC-III) April 27-30, 2016 Papillon Ayscha Hotel, Belek, Antalya, Turkey