CUKUROVA UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCES

PhD THESIS

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INVESTIGATION OF PROTEIN TYROSINE KINASE ENZYME IN BEHÇET'S PATIENTS

DEPARTMENT OF CHEMISTRY

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ABSTRACT

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Behçet disease is a chronic disease that affects many systems of the body, mucocutaneous lesions indicative of a multi system disease (oral ulcers, genital ulcers, and skin pustules),arthritis and intraocular inflammation. The pathogenesis of Behçet's disease is poorly understood but it is considered a complex polygenetic syndrome, with environmental triggers. The highest prevalence is seen in Turkey. In this Study, Protein Tyrosine Kinase Enzyme level was examined. The mean (min-max values) Protein Tyrosine Kinase Enzyme levels in 50 Behçet's patients were found as 5.57±1.20 (3.91-7.83) ng/ml and The mean (min-max values) Protein Tyrosine Kinase Enzyme levels in 50 healthy humans(control group) were found as 2.42±0.72 (1.26-3.63) ng/ml.

When the results were examined, it was seen that there was a statistically significant increase Protein Tyrosine Kinase Enzyme level of the patient group compared to the healthy control group ($p<0.0001$).

Key Words: Behçet's disease, Protein Tyrosine Kinase Enzyme

ÖZ

DOKTORA TEZİ

BEHÇET HASTALIĞINDA PROTEİN TİROZİN KİNAZ ENZİMİNİN ARAŞTIRILMASI

Akram Mudher Kareem ALJUBOURI

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Behçet hastalığı vücudun birçok sistemini etkileyen kronik bir hastalıktır, mukokutanöz lezyonlar (oral ülserler, genital ülserler ve deri püstülleri), artrit ve göz içi iltihabı ile karakterize multisistem bir hastalıktır. Behçet hastalığının patogenezi tam olarak anlaşılamamıştır ancak çevresel tetikleyicileri olan karmaşık bir poligenetik sendrom olarak kabul edilmektedir. Prevalansı en yüksek ülke Türkiye'dir. Bu çalışmada, Behçet hastalarında Protein Tirozin Kinaz Enzim düzeyleri kıyaslanmıştır. 50 hastada ortalama (min-maks değerler) düzeyi Protein Tirozin Kinaz Enzim düzeyi 5,57±1,20 (3,91-7,83) ng/ml, 50 sağlıklı insanda ortalama (min-maks değerler) düzeyi Protein Tirozin Kinaz Enzim düzeyi 2,42±0.72 (1,26-3,63) ng/ml olarak bulundu.

Sonuçlar incelendiğinde hasta grubu Protein Tirozin Kinaz Enzim düzeyinde control grubuna kıyasla istatistiksel olarak anlamlı bir artış olduğu görülmüstür ($p<0.0001$).

Anahtar Kelimeler: Behçet hastalığı, Protein Tirozin Kinaz Enzim

EXTENDED SUMMARY

Behçet's disease is chronic, inflammatory disease that is common worldwide. It affects many organs and systems. In Behçet's disease, which usually causes skin wounds; The eyes, joints, intestines, veins, brain, spinal cord and digestive system may also be affected (Yazici et al., 1999; Mat et al., 2013).

A specific way of diagnosis exists. However, the international Behçet's disease study group's criteria are used to make the diagnosis.. One of the main features of inflammation in BD is the phenomenon of pathergy, which is tissue hyperreactivity and hypersensitivity response observed after minor trauma .The phenomenon of pathergy differs from other criteria because it is more objective (Ozdemir et al., 2007).

Between 1924-1936, Turkish Doctor Hulusi Behçet observed three patients with mouth, genital ulcer and eye complaints. Based on the symptoms he observed in these patients, he thought that the findings might be related to a different disease. He published his views on this subject in the journal "Dermatologische Wochenschrift" in 1937. He also presented the same thoughts at the dermatology meeting held in Paris (Satar, 2009).

The triple symptom complex mentioned by Dr. Hulusi Behçet was included in the terminology as Behçet's Disease, and it started with Jensen (1941). At the 1947 Geneva International Medical Congress, the terms "Morbus Behçet" or "Behçet's Disease" were adopted instead of the triple symptom complex. This naming, initiated by Jensen, has become widespread all over the world (Wozniacka et al., 2015). The fact that this triple symptom complex, which Hulusi Behçet stated as a separate disease, took its place in the terminology as Behçet's disease started with Jensen (1941). In the International Medical Congress held in Geneva in 1947, the term "Morbus Behçet" or "Behçet's Disease" was adopted instead of the definition of the triple symptom complex, and this nomenclature was initiated by Jensen, became widespread worldwide (Wozniacka et al., 2015).

Studies carried out in later periods revealed that the disease is a multisystemic disease that affects many systems such as the skin, joints, gastrointestinal system, respiratory system, cardiovascular system, etc (Onder and Gurer, 2001).

In Behçet's disease, which usually causes skin wounds; the eyes, joints, intestines, veins, brain, spinal cord and digestive system may also be affected. The most common symptom of the disease is recurrent sores in the mouth. These sores, called "oral aphthae", can occur anywhere in mouth and cheek. Another common symptom of BD is sores in genital area. In addition, the findings may vary depending on the organs involved. Hard red lesions on the legs, pain in the eyes, blurred and low vision, headache, weakness in the arms and legs, bloody sputum etc can be seen. Different complications occur depending on the organ involvement in the course of BD and the agents (drugs) used in the treatment. In eye involvement, varying degrees of vision loss or blindness; In brain involvement, chronic headache, loss of strength in the arm and/or leg can be seen. Damage to the liver, heart and other internal organs due to vascular involvement; weakness, deep wounds and varicose veins due to involvement of arm and leg veins; Bloody sputum may develop due to involvement of pulmonary vessels.

Behçet's Disease manifests itself with occasional sores in the mouth, especially on the inner face of the lips. These wounds are as seen in the picture above and these wounds are painful. The bases of these wounds are white and surrounded by a red halo. These sores, which are both in the mouth and in the genital area, sometimes go away on their own and reoccur from time to time. These sores usually appear early in the disease. These wounds heal without a trace in 1-2 weeks and can come out again and again. Similarly, it is observed in the genital organs, namely on the skin of the bags in men, and on the outer surface of the vagina in women ,These sores are less common than mouth sores. These sores are larger than those in the mouth. These wounds heal more slowly and leave scars as they heal, but they recur less frequently.

Although it is a disease that affects a lot of people over the world, Turkey, Central Asian nations, and Mediterranean nations have higher prevalence rates than any other country on the Silk Road (Alpsoy, 2016).

The disease is most commonly seen between the ages of 20-40. The age of onset of the disease has been reported as 29 (Ugurlu et al., 2015). It is rarely seen in childhood. Familial BD cases are mostly seen at earlier ages. While 8% of Turkish patients have a family history, this rate is between 2-3% in Japan (Gul et al., 2000).

Although studies conducted with data obtained from hospitals showed that BD is more common in males, it has been reported that it is seen at a similar rate in both sexes as a result of recent studies (Behcet, 1937).

Gender affects both clinical findings and prognosis. While papulopustular lesions, neurological involvement, ocular findings, lung involvement, pathergy positivity and thrombophlebitis are more common in men than in women, genital ulcers, erythema nodosum and arthralgia are more common in women (Saylan, 1997). However, it is known that the disease's clinical course is more severe in young men (Tuzun, 2009).

The clinical features of the disease also differ between geographical regions. For example, gastrointestinal involvement is quite common in Japan and Korea, and this situation emerges as important causes of morbidity and mortality (Ideguchi et al., 2011; Skef et al., 2015). On the other side, Turkey and other Mediterranean nations tend to have higher rates of pathergy positive. HLA and B51 positive are more prevalent in countries in the Middle East and Far East (Sakane et al., 1999; Dalvi et al., 2012).

The prevalence of BD in Japanese living in the USA is lower than in Japanese living in their homeland (Hirohata, 1975). In Turks residing in Germany, the incidence was reported to be comparable to Turkey (77.3/100.000) (Papoutsis et al. 2006).

Autoimmune reactions are thought to cause vasculature that primarily targets vessels. Antientodelic cell antibodies have been identified in BD and many vasculitides and are thought to become active by binding to endothelial cells and increasing cytokine release. In the US, alpha enolase has been defined as the target of IgM-type AECAs, and it is thought that AECAs also have a complement- or antibody-dependent cellular toxicity-increasing effect (Aydıntug et al., 1993).

Aphthous stomatitis, the major criterion, is seen in almost 100% of patients. However, in 1% of patients, other findings of BD can be detected without oral aphthae (Kalayciyan and Arzuhal, 2005). Since oral aphthae are common in the healthy population, it is a low-sensitivity criterion for diagnosis (Kural et al., 2003).

Ulcers in the mouth are often the first sign of aphthous ulcers, and although they appear years before the diagnostic criteria are met, their frequency and severity increase with the appearance of other findings. These ulcers begin as erythematous papules, become slightly sunken from the surface after approximately 48 hours, are covered with a yellow-white pseudomembrane, and eventually turn into painful ulcers (Alotaibi et al., 2005). In the histopathology of the lesion, there is a nonspecific ulcer with necrotic material, and vasculitis findings are mild and moderate (Ghate and Jorizzo, 1999).

Although oral aphthous lesions in BD are more numerous and painful than other oral aphtha-causing diseases, they cannot be differentiated in terms of appearance and localization (Letsinger et al., 2005).

Protein Kinase Enzymes that biochemically alter proteins as a result of the addition of a phosphate group are known as protein kinases. Protein kinases, which play a major role in the creation of cellular responses such as cell proliferation and death, and the phases of intercellular signal transmission, are known to account for around 500 genes and 2% of all eukaryotic genes in the human genome (Manning and Cantley, 2002).

In this study, the Protein Tyrosine kinase enzyme level in Behcet patients was examined. This study included Behcet patients who applied between May 2020 and May 2022 to the cukurova University Faculty of Medicine, Department of Internal Medicine and Department of Rheumatology. Within the scope of the study, two groups, a control group (n = 50) and a patient group (n = 50), were established. The study data were analyzed utilizing the SPSS 21.0 Package Program. As descriptive statistics, the mean and standard deviation were reported.

As a result of the analysis of the data obtained from the study, Protein Tyrosine Kinase Enzyme level was found to be 5.57 (3.91-7.83) ng/ml in Behçet patients and 2.42 (1.26-3.63) ng/ml in healthy controls. As determined by the independent sample T-test to determine whether the Protein Tyrosine kinase levels in Behçet patients and healthy control groups differed, it was determined that Protein Tyrosine Kinase level (5.57±1.20 ng/ml) and Protein Tyrosine kinase level in Behçet patients were significantly higher than in the healthy controls (2.42±0.72 ng/ml)($p<0.001$).

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TABLE OF CONTENTS **PAGE**

LIST OF TABLES **PAGE**

LIST OF FIGURES **PAGES**

LIST OF ABBREVIATIONS

ADP : Adenosine diphosphate
ATP : Adenosine triphosphate
BC : Before Christ
BD : Behçet's disease
BDCAF : Behçet's disease current activity form
CNS : Central nervous system
CRP : C-reactive protein
DAIBD : Disease activity index for intestinal Behçet's disease
ESR : Erythrocyte sedimentation rate
HLA : Human leucocyte antigen
HSP : Heat shock protein
IBDDAM : Iranian Behçet's disease dynamic activity measure
ICBD : International criteria for Behçet's disease
ISG : International Study Group
NRTKs : non-receptor tyrosine kinases
PH : pleckstrin homology
PPP : phospho-protein phosphatase
PTB : phospho-tyrosine binding
PTK : Protein tyrosine kinases
PTM : Post-translational modification
PTP : phospho-tyrosine phosphatase
RPTK : Receptor protein tyrosine kinases
TEC : Tyrosine kinase expressed in hepatocellular carcinoma
TNFR : Tumour necrosis factor receptor
UK : United Kingdom

1. INTRODUCTION

1.1. Behcet's Disease

1.1.1. Definition

Behcet's disease is an inflammatory disorder that affects many parts of the body. It most often affects the joints, skin, central nervous system (CNS), and digestive tract, and it is marked by mouth ulcers, vaginal ulcers, and inflammation of the eyes.It is categorized as a systemic vasculitis and can affect practically any organ's arteries and veins. The cause of Behcet's disease is still unknown, however the most accepted theory about its pathogenesis holds that an infectious agent causes a significant inflammatory response in a genetically vulnerable host (Behcet, 1937) In Behçet's disease, which usually causes skin wounds; the eyes, joints, intestines, veins, brain, spinal cord and digestive system may also be affected. The most common symptom of the disease is recurrent sores in the mouth. These sores, called "oral aphthae", can occur anywhere in mouth and cheek. Another common symptom of BD is sores in genital area. In addition, the findings may vary depending on the organs involved. Hard red lesions on the legs, pain in the eyes, blurred and low vision, headache, weakness in the arms and legs, bloody sputum etc can be seen. Different complications occur depending on the organ involvement in the course of BD and the agents (drugs) used in the treatment. In eye involvement, varying degrees of vision loss or blindness; In brain involvement, chronic headache, loss of strength in the arm and/or leg can be seen. Damage to the liver, heart and other internal organs due to vascular involvement; weakness, deep wounds and varicose veins due to involvement of arm and leg veins; Bloody sputum may develop due to involvement of pulmonary vessels.

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and other internal organs due to vascular involvement; Weakness, deep wounds and varicose veins due to involvement of arm and leg vessels, bloody sputum may develop due to involvement of pulmonary vessels.

Behçet's Disease manifests itself with occasional sores in the mouth, especially on the inner face of the lips. These wounds are as seen in the picture above and these wounds are painful. The bases of these wounds are white and surrounded by a red halo. These sores, which are both in the mouth and in the genital area, sometimes go away on their own and reoccur from time to time. These sores usually appear early in the disease. These wounds heal without a trace in 1-2 weeks and can come out again and again. Similarly, it is observed in the genital organs, namely on the skin of the bags in men, and on the outer surface of the vagina in women, as seen in the picture below. These sores are less common than mouth sores. These sores are larger than those in the mouth. These wounds heal more slowly and leave scars as they heal, but they recur less frequently.

Different complications occur depending on the organ involvement in the course of BD and the agents (drugs) used in the treatment. In eye involvement, varying degrees of vision loss or blindness; In brain involvement, chronic headache, loss of strength in the arm and/or leg can be seen. Damage to the liver, heart and other internal organs due to vascular involvement, weakness due to involvement of arm and leg vessels, deep wounds and varicose veins, bloody sputum due to involvement of pulmonary vessels may develop (Kural et al., 2003). GH duration and frequency show unpredictable remissions and exacerbations (Alpsoy et al., 2007).

1.1.2. Historical Background

Turkish Doctor Hulusi Behçet followed 3 patients with oral, genital ulcer, and eye complaints about a long time between 1924-1936 and thought that the findings might be related to a different disease based on the symptoms of these

patients. He published his views on this subject in the journal "Dermatologische Wochenschrift" in 1937 (Satar, 2009).

The fact that this triple symptom complex, which Hulusi Behçet stated as a separate disease, took its place in the terminology as Behçet's disease started with Jensen (1941). In the International Medical Congress held in Geneva in 1947, the term "Morbus Behçet" or "Behçet's Disease" was adopted instead of the definition of the triple symptom complex, and this nomenclature was initiated by Jensen, became widespread worldwide (Wozniacka et al., 2015).

Studies conducted in the following years revealed that BD is a multisystemic disease includes skin, joint, neurological, vascular, gastrointestinal, pulmonary, and cardiac effects in addition to this triple finding (Onder and Gurer, 2001).

Figure 1.1. Dr Hulusi Behçet (source :www.tiphastaligi.com)

1.1.3. Epidemiology

Behcet's disease has a very specific geographic distribution. It is seen most commonly along the Silk Road, which is an old trading route that connects the Mediterranean with East Asia. Along this route, it is one of the primary causes of morbidity (Idil et al., 2002). It is estimated that between 110 and 420 cases of the condition are diagnosed per one hundred thousand people in Turkey, which is the nation with the highest occurrence of the condition. (Idil et al., 2002; Azizlerli et al., 2003), whereas it is 13–20 per 100 000 in Japan and 1–2 per 100 000 in the United Kingdom and the United States (Sakane et al., 1999). It has never been heard of among Ameri-Indians, and people of African descent have rarely reported it (Jacyk ,1994 ; Poon et al., 2003) .

Behcet's disease is uncommon in childhood and often manifests in adults (Kone-Paut et al., 1999). Men in eastern Mediterranean nations are more likely to contract the illness and experience its more severe manifestations. (Tursen et al., 2003). In Asian populations, the gender ratio is inverted (Bang et al., 1997). In the Caucasian population, familial sickness is very uncommon; nonetheless, a positive family history has been documented in up to 12% of non-caucasoid individuals. (Kone-Paut et al., 1999), and a Turkish study revealed a sibling risk ratio of 11.4–52.5. (Gul , 2000) This shows that the disease shares a hereditary component inflammatory bowel disease and other complex genetic abnormalities

The age range where the disease is most common is between 20-40 years, and The average age of illness onset is 29 (Ugurlu et al., 2015). The disease is most commonly seen sporadically. Familial BD cases are usually seen at earlier ages. While 8% of BD patients in Turkey have a positive family history of BD, this rate varies between 2-3% in BD patients in Japan (Gul et al., 2000).

Although it was initially reported that BD is more common in male patients in studies based on hospital data, the studies have shown that the disease is seen equally in both sexes (Behcet, 1937).

In addition to clinical signs and symptoms, prognosis also differs according to gender. Papulopustular lesions, ocular findings, neurological involvement, pulmonary involvement, pathergy positivity and thrombophlebitis are more frequent in males. On the other hand, genital ulcers, erythema nodosome, and arthralgia were reported to be more frequent in females (Saylan, 1997; Tuzun, 2009).

1.1.4. Etiopathogenesis

There are no clear data on what causes the disease. However, it is thought that many factors such as genetic, environmental, immune system, etc. have an effect on the emergence of the disease.

Considering all the data, the etiopathogenesis of BD is a vasculitis that has not been fully elucidated. In people who are genetically predisposed, there is a deterioration in the immune system in the presence of certain environmental triggers (Kose, 2009; Hedayetfar, 2013).

BD is generally defined as vasculitis involving veins and arteries (Kose, 2009; Hedayetfar, 2013). Venous involvement is the prominent feature of the disease and BD is differentiated from other vasculitides mainly by venous involvement and tendency to thrombosis. It is noted that vascular involvement and thrombosis in BD may result from endothelial damage and endothelial dysfunctions (Kose, 2009; Hidayetfar, 2013).

1.1.4.1. Infectious Agents

BD is not an infectious disease, but infectious agents are responsible for its triggering and development as environmental factors. T cell-mediated immune response against Herpes simplex virus (HSV) was found to be impaired in Behçet's patients. It is thought that hepatitis C virus and parvovirus B19 infection may also have an effect on the pathogenesis of BD. However, there is not enough data on

this subject yet. In the etiopathogenesis of BD, especially streptococcal infections (S.sangius, S.fecalis, S.pyogenes, S.salivarius) are emphasized. Immune system dysfunction after infection is blamed for the emergence of BD rather than the direct effects of these infections (Dabbagh et al., 2014).

Studies that started with the suspicion of Doctor Hulusi Behçet that a virus could be found in the etiology of the disease and questioning the role of microbial agents in the pathogenesis continues without slowing down today (Behcet, 1937). Many studies have been conducted on bacteria (Streptococcus, Staphylococcus, Borrelia, Helicobacter, Mycoplasma, Escherichia coli), viruses (Herpes simplex, Cytomegalovirus, Varicella, Hepatitis, Parvovirus b19), fungus (Candia) and parasite (Trichomonas vaginalis). Although the data on the role of herpes simplex virus, Streptococcus sanguinis, and other streptococcal antigens in the etiology is strong, this is controversial for other microbial agents (Dabbagh et al., 2014).

The S.sanguinis antigen carries a similar amino acid sequence to the heat shock proteins (HSP) expressed on the cell surface in conditions such as microbial stimuli and physiological stress on the cell membrane (Mendoza et al., 2010).

HSP-65 from bacteria has broadly similar homology to human HSP-60. In addition, there is over 50% homology between mycobacteria and human HSP (Lehner, 1997).

In studies, S.sanguinis and HSP activate 60/65 kDa T cells in Behçet's patients, but this does not occur in the control group. Based on this data, it was reported that repetitive bacterial stimulus increased the expression of HSPs in mucus-secreting cells and reactive anti-mucous T cells caused damage in genetically susceptible individuals, and this situation was called the "molecular mimicry model" (Kaneko et al., 2008).

1.1.4.2. Genetic Factors

Genetic susceptibility 6p21 HLA complex is the most extensively researched genetic locus for Behc et's disease. Consistently, disease risk has been

linked to variants in the HLA-B gene, namely HLA-B*51. (Ohno et al,1982). All racial and ethnic groups have corroborated this, however the association is bigger among Turkish and Japanese illnesses than among Caucasians. (Verity et al., 1999). There are at least 34 allelic variants of HLA-B*51; the relationship has been narrowed down Regarding the two molecular subtypes that are found the most frequently, HLA-B*5101 and HLA-B*5108 (Kotter et al.,2001). HLA-B*57 carries a disease risk comparable to HLA-B*51 in Caucasians. (Ahmad et al.,2003).

Unknown is the molecular mechanism by which certain HLA-B alleles cause illness. It's unclear if these associations are primary or if risk alleles are in linkage disequilibrium. Both MICA*009 (Ota et al., 1999) and TNF-1031C(Ahmad et al., 2003) are found on the HLA-B*5101 haplotype (Ahmad et al.,2003). Moreover, these relationship is not permanent: The Disease risk linked with HLA-B*51 wide-ranging among racial groups, and maladies alleles are prevalent in those communities where the disease is essentially unknown (Verity et al., 1999).

A Genome scan found another vulnerability region on chromosome 6p (Gul et al.,2001). Polymorphisms in additional potential genes besides HLA studied, coagulation factor V, (Toydemir et al.,2000 ; Gurgey et al., 2003), endothelial nitric oxide synthase (Salvarani et al., 2002), and intercellular adhesion molecule 1 (Salvarani et al., 2002; Boiardi et al.,2001; Verity et al.,2000).

Nevertheless, many of these trials were conducted on a small number of patients, and positive outcomes have not been replicated. Therefore, the significance of other genetic variants to illness risk is questionable.

Although there are important data showing that the disease is not a hereditary disease, it can be said that genetic predisposition according to the findings of the studies, plays a significant role in the emergence of the disease.As a result of the researches, it has been shown that the tissue antigen named HLA-B51 is seen in the majority of the patients. However, the HLA-B51 antigen is widely

seen in the community. Therefore, it cannot be said that only this antigen is responsible for the emergence of the disease.

The major tissue compatibility complex (MHC), also known as human leukocyte antigens (HLA), is the name given to the molecules responsible for antigen presentation on the cell surface and the group of genes that encode them.

Studies have shown that the HLA B5 and B51 alleles is one of the most important risk factors for BD patients (Dementhon et al., 2009).

The presence of HLA-B51 allele in the Far East and Mediterranean countries, where BD is quite common, is 20-25% in the normal population, while this rate varies between 55-65% in BD patients. The relative risk of the presence of the relevant allele for the occurrence of BD in these countries was found to be in the range of 6-13 (Houman et al., 2013).

In Western Europe and the USA, the presence of HLA-B51 is seen at a lower rate both in the healthy population and in Behcet's patients, and the presence of this allele has a weaker relationship with the disease (Gul et al., 2001; Ohno et al., 1982; Krause et al., 2009).

As a result of studies, it has been reported that some MHC-1 and MHC-1-related sequence a (MICA) single-nucleotide polymorphisms are also associated with BD (Remmers et al., 2010).

In addition, several genes encoding HLA-A, B, and C subtypes have been linked to BD, according to studies.and genes encoding some HLA-A and B subtypes may also have an inhibitory effect on the formation of the disease (Wallace, 2014). Although the mechanism by which HLA molecules play a role in the formation of Behcet's disease is not clear, it is thought that their interaction with lethal immunoglobulin-like receptors in NK cells triggers NK cell-mediated cellular cytotoxicity and neutrophil activation, and that antigen presentation to $CD8^+$ T cells via HLA molecules also stimulates immunity (Ombrello et al., 2014; Hatemi et al., 2015a).

In various studies, cytokines such as tumor necrosis factor (TNF)-α, interlocking (IL)-1, IL-10, cytokine receptors such as IL-23R/IL-12RB2, CCR1, CCR3, primarily tumor necrosis factor (TNF)-α located in a close genetic locus with MHC-I (Xavier et al., 2012; Liang et al., 2013; Hatemi et al., 2015b).

The incidence of these polymorphisms differs between various populations. Genetic studies indicate that multiple factors predispose to Behcet's disease, and the most prominent of these is the 'MHC-I locus', which includes HLA and TNF-α genes, as well as possibly other unidentified susceptibility genes (Song and Kang, 2012).

1.1.5. Clinical Manifestations

Behçet's disease clinical manifestations vary considerably among patients, in different geographical regions, and at different stages of the disease (Kural et al., 2003). GH duration and frequency show unpredictable remissions and exacerbations (Alpsoy et al., 2007).

1.1.5.1. Systemic Involvement

- **Gastrointestinal system involvement**

The involvement of the gastrointestinal system varies greatly between populations, with Japan having a significantly higher incidence than Turkey (Sakane et al., 1999; Yurdakul et al.,1996). In addition to anorexia, vomiting, dyspepsia, diarrhoea, and abdominal discomfort, the spectrum of clinical symptoms is extensive. Indeed, Behc et's disease shares many of the characteristics of inflammatory bowel diseases, and the gastrointestinal inflammation is typically similar to that of Crohn's disease, with segmental mucosal inflammation and punched-out, fissuring, or aphthoid ulcers, most often localized in the ileocaecal region (Bayraktar et al., 2000). Transmural inflammation and fistulae are more prevalent than strictures (Kyle et al.,1991). Granulomata, the hallmark of Crohn's

disease, have been described sporadically, and Behc et's disease is a crucial differential diagnosis factor in the evaluation of inflammatory bowel disease.

Gostrointestinal involvement (GI) of BD is called 'Intestinal BD' and 'Entero-Behçet'. In BD, involvement can be seen in the entire GI tract from the lips to the anus. GI involvement is most commonly observed in the ileocecal region and colon. In clinical practice, esophageal ulcer is characterized by ulcers in stomach, duodenum, ileum and jejunum. There may also be hemorrhagic ulcerations in the colon. The frequency of gastrointestinal involvement, which is often confused with inflammatory bowel diseases and causes significant symptoms such as abdominal pain or bleeding, varies between geographical regions. The frequency, which is 2.8% in Turkey, was found to be 38-52% in England (Temiz, 2020). Gastrointestinal involvement is seen at a rate of 60% in Japan (Marshall, 2004).

- **Ocular Involvement**

30–70% of people with Behcet's illness have problems with their eyes. and is more prevalent and severe in men than in women (Sakane et al., 1999; Tursen et al., 2003; Kural-Seyahi et al., 2003) (Tursen et al.,2003; Kural-Seyahi et al.,2003). Ocular illness is typically bilateral and typically manifests within two to three years of disease initiation (Kural-Seyahi et al.,2003). Ten to twenty percent of patients present with this symptom Chronic, relapsing bilateral uveitis of the anterior and posterior uveal tracts causes significant morbidity. Anterior uveitis with hypopyon, in which inflammatory exudate generates a visible layer of cells in the anterior chamber, is a classic indicator of ocular Behcet's illness. In combination with posterior uveitis and retinal vasculitis, this can cause vision loss in up to 25% of patients, however immunosuppressants are improving the prognosis. (Muhaya et al.,2000). Iridocyclitis, scleritis, keratitis, vitreous hemorrhage, optic neuritis, retinal vein blockage, and retinal neovascularization are some other types of eye damage (Verity et al.,2003). The counterintuitive sparing of the conjunctiva and

absence of respiratory mucosal lesions may imply a function of gastrointestinal bacteria in infection onset and maintenance (Verity et al.,2003).

Eye involvement has a special importance as it causes high morbidity in Behçet's patients. Eye involvement can be seen in 29-80% of patients. Ocular involvement in Behçet's disease generally occurs 2 years after aphthous ulcer. However, it can be seen as the first finding in 10-20% of the patients. Since some findings in ocular involvement of BD are pathognomonic, there are also studies on ocular involvement alone that can make a diagnosis. The incidence of BD-related uveitis is higher in males than in females, and the course of the disease is more aggressive in males. The disease progresses more mildly in elderly and female patients.

Complications that may result in permanent vision loss are observed as a result of recurrent attacks and the drugs used in the treatment of these attacks. These are optic disc pallor, optic atrophy, scarring of the macula, retinal scar, secondary glaucoma, cataract, and retinal detachment (Evereklioglu and Duzgun, 2017). Especially in young males, more frequent and severe involvement is seen and may result in blindness. Again, in this patient group, it tends to involve the posterior segment more. Posterior uveitis is the most characteristic ocular finding. Anterior uveitis, hypopyon, secondary glaucoma, cataract, conjunctivitis, scleritis, keratitis, vitreous hemorrhage and optic neuritis may also be seen. Blurring of vision, pain in the affected eye, discomfort from the sun and light, vision loss may occur in eye involvement due to BD. The eye is one of the most important areas of involvement in BD. Therefore, every diagnosed patient should be followed up regularly in the eye diseases department (Calamia et al., 2005; Davis and Moschella, 2018). Observation of retinal vasculitis is in favor of severe involvement.

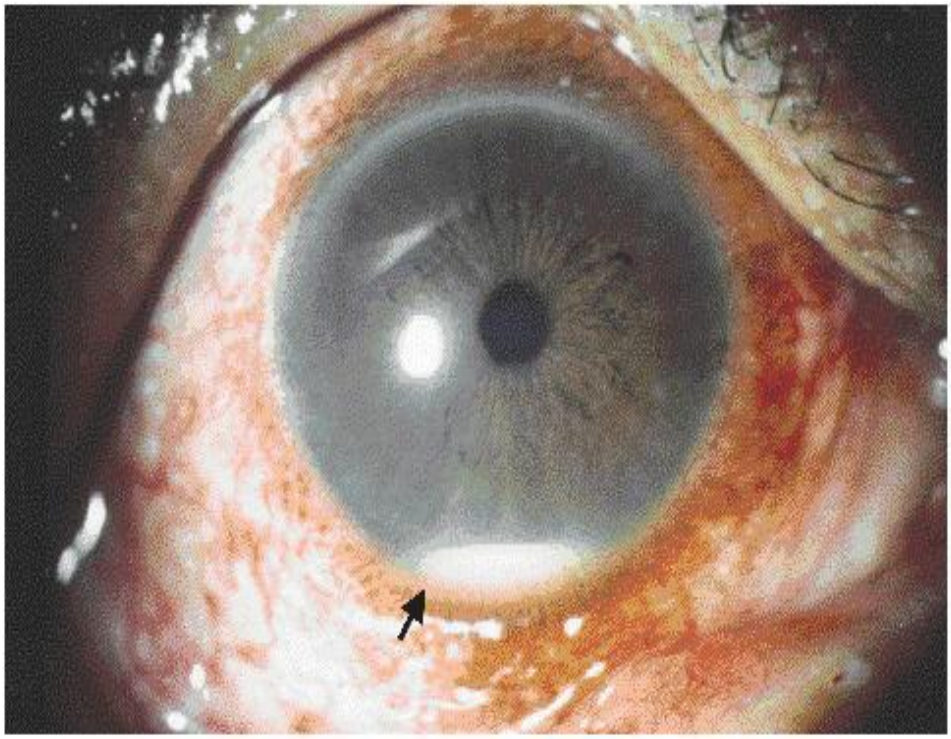


Figure 1.2. Hypopyon in BD patient (Ramsay and Lightman, 2001).

- **Vascular involvement**

Systemic vasculitis Behcet's disease affects both the arteries and veins. Most of the pathological process of disease is caused by small-vessel vasculitis, and 7 to 49% of individuals have clinically obvious large-vessel involvement (Sakane et al., 1999; Kural-Seyahi et al.,2003). Deep venous thrombosis and superficial thrombophlebitis can both develop from venous involvement, which is more prevalent (Kuzu et al.,1994). Additionally occurring and carrying a dismal prognosis are vena cava, dural sinus, and Budd-Chiari thrombosis. Venous thromboses are connected to both arterial aneurysms and occlusions (Hamuryudan et al.,1994). A significant cause of mortality, pulmonary artery aneurysms was found in 1% of Turks (Hamuryudan et al.,1994). All but one of these 24 patients presented with haemoptysis.Cardiac involvement is uncommon, but it has been

reported Pericarditis, valve defects, thrombosis, fibrosis, and coronary artery involvement can develop. The coagulation cascade is not specifically defective in Behcet's illness. Anti-cardiolipin antibodies, for example, are known thrombophilic factors that can cause intravascular clotting but are not specifically linked to thrombo-embolic complications (Mader et al., 1999; Espinosa et al., 2002).

- **CNS involvement**

CNS involvement occurs in 5–10% of cases (Sakane et al., 1999). Neurological signs often appear within five years of the initiation of the disease and are more prevalent in male. Brain parenchyma involvement is prevalently used (80%), affecting the medulla in particular (Akman-Demir et al.,1999). Also possible are non-parenchymal diseases such as dural sinus thrombosis, aseptic meningitis, and arterial vasculitis. Symptoms vary, however, frequent are bilateral symptoms, hemiparesis, behavioral abnormalities, migraines, and dysplasia (Akman-Demir et al.,1999). Typically, Normal cerebrospinal fluid may have more neutrophils, protein, and pressure. Neurological sickness is morbid. (Siva et al., 2001), and the fatality rate is typically considered to be between 5 and 10%. (Akman-Demir et al.,1999; Siva et al.,2001).

1.1.5.2. Skin and Mucous Involvement (Mucocutaneous Involvement)

Mucocutaneous findings in BD include oral and genital ulcers, erythema nodosum, papulopustular lesions, extragenital ulcers, and thrombophlebitis (Alotaibi et al., 2005).

- **Genital ulcers**

72–94% of people get genital ulcers. (Sakane et al., 1999; Kural-Seyahi et al.,2003) and resemble mouth ulcers morphologically, but often heal by scarring. In males, scrotum lesions are more prevalent, whereas penile lesions are infrequent. Epididymitis is also prevalent, although urethritis is not a characteristic of Behcet's

illness, which can be used to differentiate it from Reiter's syndrome. Ulcers on the vulva, vagina, and cervix may cause dyspareunia in females. Perineal, perianal, and groin ulcers affect both sexes.It is seen between 57-93% in different societies (Alpsoy et al., 2007). Although genital lesions are rarer than oral lesions, they are larger, painful, have irregular borders, and tend to leave scarring. Detection of these genital scars on examination, even if there is no active ulcer, is diagnostically important (Kural et al., 2003). They are most common in the scrotum (90%) in men and most commonly in the labia in women. Viral culture, direct fluorescent antibody staining, or PCR examination may be necessary to differentiate lesions from genital herpes ulcers. While the most common involvement in men is observed in the scrotum, it is most frequently observed in the labia in women. It is oval and round shaped. Although it resembles oral aphthae in clinical appearance, it is more deeply located and heals with scar tissue. Thanks to this feature, even if active ulcer is not seen, it gives an idea about previous genital ulcer (Alpsoy et al., 2007).

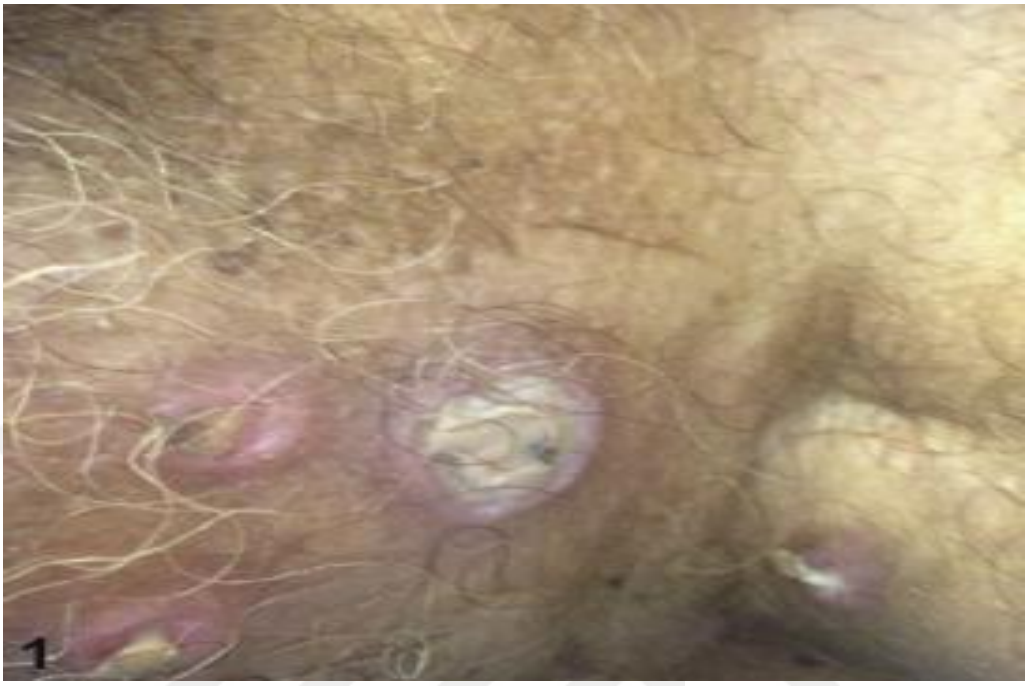


Figure 1.3. Appearance of genital ulcer in a male patient (Curtiss et al., 2018)

- **Erythema nodosum-like lesions**

These lesions are more frequent in females and be 15-78% in studies (Gul et al., 1999). Lesions are often located on the legs and thighs.

Although these lesions are clinically similar to erythema nodosum seen in the course of systemic diseases, they are histopathologically different, therefore they are defined as erythema nodosum-like lesions. In contrast to classical erythema nodosum, vasculitis and vascular reaction are prominent in its histopathology (Kim and Leboit, 2000).

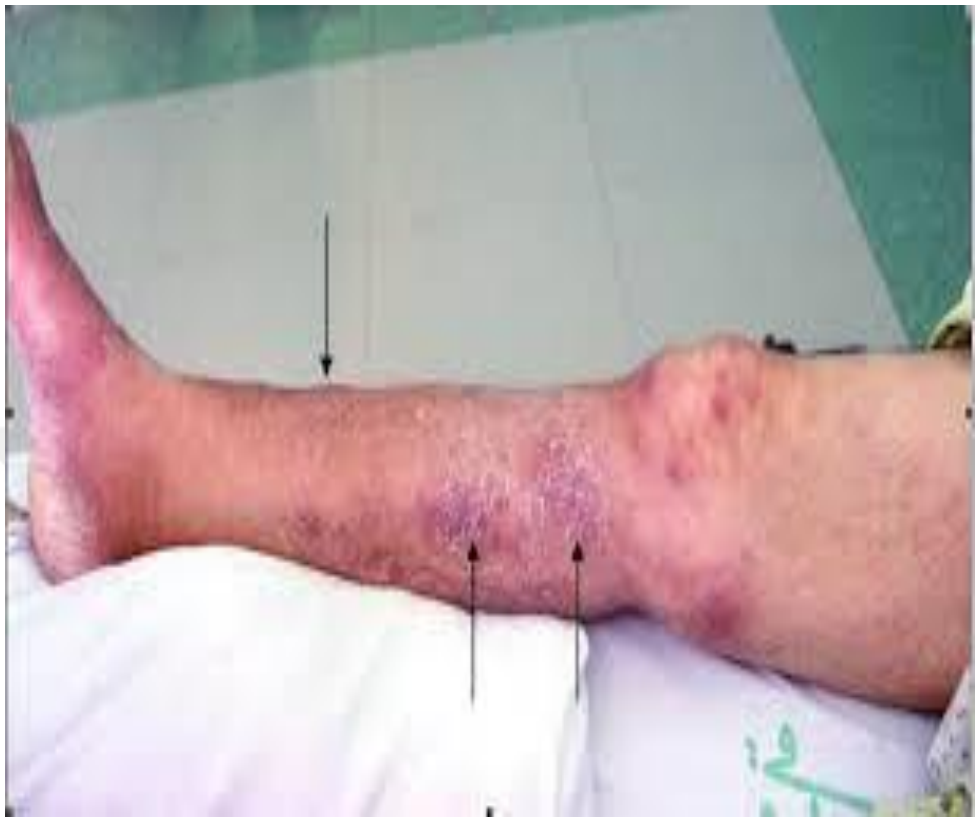


Figure 1.4. Appearance of Erythema nodosum-like lesion lesions in a BD (Nouri-Majalan and Moghimi, 2008).

- **Oral aphthous ulcers**

Behçet's disease requires chronic aphthous ulceration. Oral ulcers are often the first sign of a disease, years before systemic symptoms appear. Oral ulcers resemble conventional mouth ulcers in appearance and location, but they can be more painful and grow from a flat ulcer to a large sore. Individual or group lesions heal without scarring. Oral ulcers usually affect the tongue, lips, gingiva, and buccal mucosa, although they can also affect the palate, throat, and tonsil. Oral ulcers can be little (10 mm in diameter, shallow, encircled by an erythematous halo, healing without scarring), major (morphologically similar but larger, more painful, more persistent, and may leave a scar), or herpetiform (recurrent crops of

hundreds of small and painful ulcers that may become confluent). Dental operations or trauma often induce mouth ulcers. Without systemic illness, smoking is linked to repeated aphthous ulcers (Shapiro et al., 1970).

Aphthous stomatitis, the major criterion, is seen in almost 100% of patients. However, in 1% of patients, other findings of BD can be detected without oral aphthae (Kalayciyan and Arzuhal, 2005). Since oral aphthae are common in the healthy population, it is a low-sensitivity criterion for diagnosis (Kural et al., 2003).

Oral aphthous ulcers are generally the first sign of the disease, and although they appear years before the diagnostic criteria are met, their frequency and severity increase with the appearance of other findings. These ulcers begin as erythematous papules, become slightly sunken from the surface after approximately 48 hours, are covered with a yellow-white pseudomembrane, and eventually turn into painful ulcers (Alotaibi et al., 2005). In the histopathology of the lesion, there is a nonspecific ulcer with necrotic material, and vasculitis findings are mild and moderate (Ghate and Jorizzo, 1999).

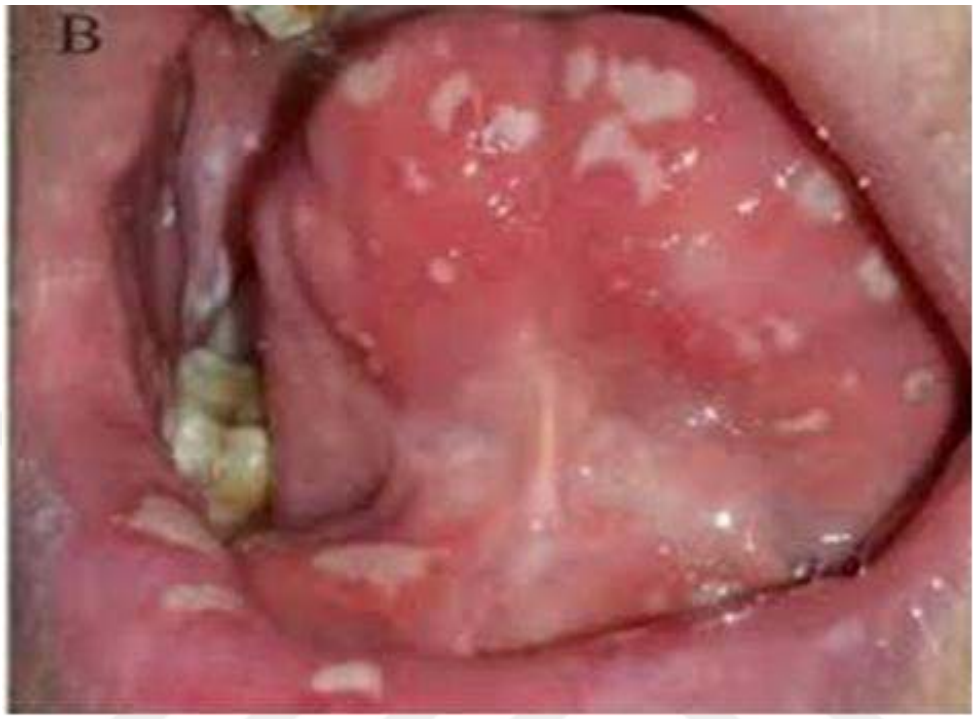


Figure 1.5. Oral ulcerations (Nur'aeny, 2015)

Although oral aphthous lesions in BD are more numerous and painful than other oral aphtha-causing diseases, they cannot be differentiated in terms of appearance and localization (Letsinger et al., 2005). In addition, approximately 52.2% of patients with recurrent aphthous somatitis were diagnosed with BD over time, which should not be ignored (Bang et al. 1997).

Oral aphthae seen in almost all patients are superficial lesions of varying diameters, oval-round shaped, surrounded by an erythematous halo, and covered with a yellow-gray pseudomembrane. The most common sites are the tongue, lips and cheek mucosa. Recurrence at least three times within a year is considered the most important criterion in diagnosis (Letsinger et al., 2005).

There are 3 types of aphthae:

1. Minor aphthae: It is the most common type of aphtha. They do not leave scars (Letsinger et al., 2005).

2. Major aphthae: They are lesions larger than 0.5 cm and heal by scarring in more than 15 days. Major aphthae occur in approximately 10% of BD patients. They are frequently located on the buccal mucosa, sublingual, lips, hard palate and tonsils (Letsinger et al., 2005).

3.Herpetic aphthae: They are most commonly located on the palate, gums and the back of the tongue (Letsinger et al., 2005).

- **Papulopustular lesions**

It is the most common skin involvement of BD after mucosal lesions. These lesions are more frequently seen in men but are seen in 28-96% of patients (Ambrose and Haskard, 2013). These lesions have been reported most commonly in patients with arthritis and positive pathergy test. Although they do not have a folliculocentric location, acneiform papulopustular can be confused with acne vulgaris lesions (Alpsoy et al., 1998).

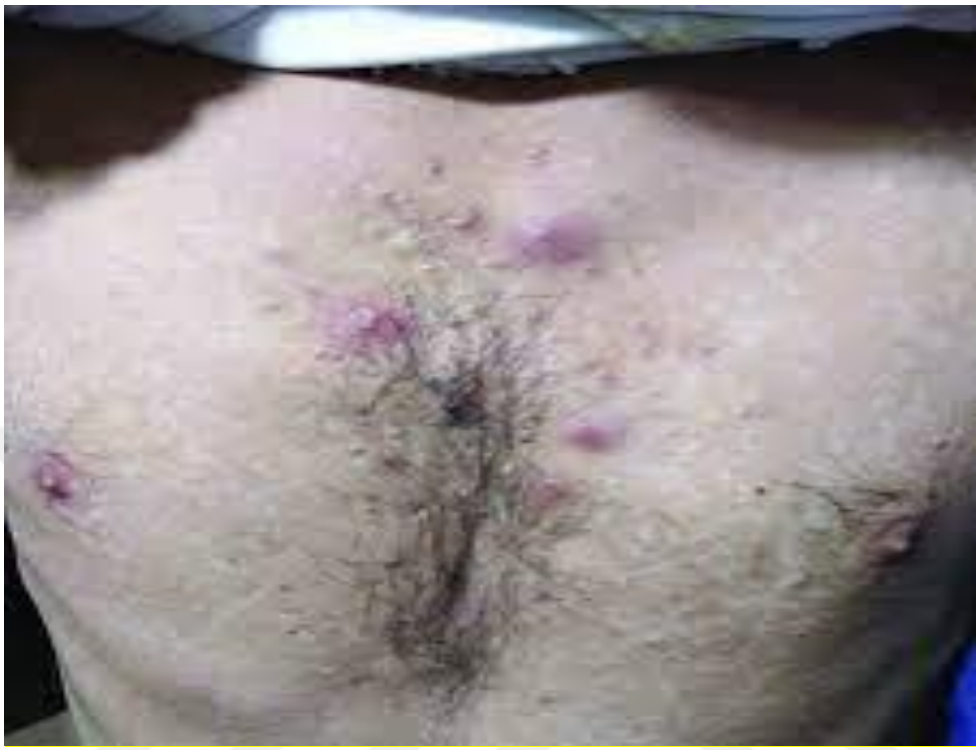


Figure 1.6. Appearance of populopustular lesions in a BD (Abd El Latif et al., 2020).

1.1.6. Diagnosis

Laboratory findings in Behçet's disease are non-specific. Neutrophil leukocytosis is observed in 15% of patients with moderate anaemia caused by a chronic illness. Non-specific immunoglobulins may rise. Rheumatoid factor, anti-neutrophil cytoplasmic antibody, and anti-nuclear antibody are negative. Nonspecific inflammatory markers like CRP and ES may be normal despite active orogenital, ophthalmic, or CNS disease (Muftuoglu et al.,1986). Due to the lack of sensitivity of the relationship with HLA-B*51, HLA typing is often ineffective in a diagnostic context.

Although there is no clear method for the diagnosis of the disease, the evaluation of the complaints of the patients is the most important process in making the diagnosis. The diagnosis of the disease is made according to the criteria

presented by the international Behçet's disease working group. Pathergy, which is tissue hyperactivity and hypersensitivity observed after minor trauma, is one of the most basic features of the disease (Ozdemir et al., 2007). Pathergy is one of the four basic minor criteria used in the diagnosis of the disease and differs from other criteria because it is more objective. Pathergy skin test is important for diagnosis. It is done by puncturing the inner surface of the forearm three times with a sterile needle. It is very painful and the reaction of the body is evaluated after 24-48 hours. This extreme skin reaction can also be seen at blood draws or operation sites. Therefore, unnecessary interventions should be avoided in patients with Behçet's disease. Some blood tests are used in the differential diagnosis of the disease, but there is no specific laboratory test for the disease. In general, tests show that the inflammation is slightly increased. Moderate anemia and increased white blood cell count can be detected. If the patient is not followed up for disease activity and drug side effects, there is no need to repeat these tests. Various imaging methods are used for children with vascular and nervous system involvement. However, an increase in CRP level and erythrocyte sedimentation rate may occur in active disease (Stone et al., 2012). The diagnosis is made by considering the systemic findings (Gonul et al., 2019).

The classification criteria published in 1990 by the International Working Group on Behçet's Disease (ICBD) include 5 findings, which are presented in Table 1.1. The sine qua non-major criterion is oral ulcers that recur at least three times a year. The other four criteria are: “genital ulcer, eye lesions (anterior uveitis, posterior uveitis, retinal vasculitis), skin lesions (erythema nodosum, pseudofolliculitis, papulopustular lesions), and positive pathergy test”. If the major criterion is accompanied by two of the other four criteria, it is considered sufficient for the diagnosis of BD. The sensitivity of the ISG criteria was 92% and the specificity was 97% (Gonul et al., 2019).

Table 1.1. Behçet's Disease International Study Group Diagnostic Criteria-1990

Recurrent oral ulceration	Minor aphthae, major aphthae or herpetiform ulcers observed by physician or patient at least 3 times a year
In addition to oral ulcers, 2 of the following criteria	
Recurrent genital ulceration	Aphthous ulceration or scarring observed by physician or patient
Ocular lesions	Anterior uveitis, posterior uveitis and vitreous cells in slit lamp examination or evaluation of the presence of renital vasculitis by an ophthalmologist.
Skin Lesions	Observation of erythema nodosum by the patient or physician, observation of pseudofolliculitis, papulopustular lesions or acneiform nodules in post-adolescent patients who do not use corticosteroids by the physician
Positive Pathergy test	Evaluation between 24-48 hours

Reference: International Study Group for Behçet's Disease (1990). Criteria for diagnosis of Behçet's disease, *Lancet*. 335:1078-1080

Although the ISG criteria are highly specific, it can be observed that patients can not be diagnosed or treatment is delayed due to the fact that oral aphtha is the major criterion and no oral aphthae are seen in 1-10% of patients (Gonul et al., 2019).

In 1993, Iran revised its diagnostic criteria by removing the mandatory requirement of oral aphthae in the ISG criteria. In the new diagnostic criteria, two points were given for oral aphthae and one point for other findings, and it was deemed appropriate to evaluate patients with a total of 3 points as BD. In addition, the classification regression tree method, which includes the method of forming subgroups for patients with insufficient findings in diagnosing BD, was used. Five subgroups used in this method are: “oral aphtha, genital ulcer, oral aphtha, skin

lesions, positive pathergy test, oral aphtha, eye findings, genital ulcers,ocular findings and positive pathergy test eye findings" (Gonul et al., 2019).

In 2004, the 11th International Conference on Behçet's Disease was held in Antalya, and in this conference, it was decided to revise the diagnostic criteria by 27 countries with the participation of Turkey. Between January 2005 and June 2006, 2556 Behçet's patients who met any of the diagnostic criteria or were diagnosed with the decision of a physician were selected as a control group, and among the 6 diagnostic criteria, 2 points in the presence of genital ulcer, eye signs, oral aphthae, presence of skin lesions, pathergy test positivity was sufficient for the diagnosis of BD. In the final criteria, 1 point was given to the classical criteria for the presence of neurological findings and the presence of oral aphthae was increased to 2 points. It was recommended to add 1 point in the presence of the pathergy test positivity, and it was stated that 4 points and above were sufficient for BD. The sensitivity of the ICBD diagnostic criteria was 94.8% and the specificity was 90.5% in its final version (Table 1.2) (Gonul et al., 2019).

Table 1.2. International Conference Diagnostic Criteria for Behçet's Disease

Manifestations	Score
Oral Aphthae	2
Genital Ulcers	2
Ocular Lesions	2
Skin Lesions	1
Vascular Manifestations	1
Neurological Manifestations	1
Positive Patergy Test*	1
*1- Pathergy test is optional. Extra points can be given when it is found positive when examined. 2- If a patient scores 4 points or more, it is defined as BD.	

Reference: International Study Group for Behçet's Disease (1990). Criteria for diagnosis of Behçet's disease, *Lancet*. 335:1078-1080

1.1.7. Prognosis

Behçet's disease is characterized by relapses and remissions are common, and the disease's clinical history is highly varied. In general, the disease is more severe in Mediterranean and Eastern populations than in Western ones, and in males as opposed to womens . Recent study of a twenty-year research of 387 Turkish individuals with Behcet's illness indicated an overall death rate of 9.8% (Kural-Seyahi et al.,2003). This is higher than previously documented (Yazici et al., 1984) likely reflects long-term follow-up in this population and illness severity. Young males had the highest rates of illness and mortality, consistent with earlier research. Intriguingly, overall mortality reduced over time, which is consistent with the notion that Behçet's disease has a generally favorable prognosis after the initial years. The primary causes of permanent disability are blindness and neurological illness.

BD is a disease that progresses with attacks and remissions and has a more severe course, especially in the male, young, and Middle Eastern or Far Eastern patients (Park et al., 1993; Yazici et al., 1996). In some patients, especially in those with mucocutaneous or joint involvement, disease activation decreases over time and remains asymptomatic for many years (Yazici et al., 1990). Neurological, ocular, and great artery or vein involvement (pulmonary involvement, bowel perforation, gastrointestinal bleeding, superior/inferior vena cava syndrome, and cerebrovascular disease) is associated with high mortality and morbidity. In a study involving 387 BD patients in Turkey, it was found that large vessel, and central nervous system involvements occur 5-10 years after the onset of the disease, while other involvements decrease over time (Yazici et al., 1990).

1.1.8. Treatment

Although the number of randomized controlled studies for Behc et's disease is on the rise, the majority of treatments are still based on empirical evidence, and there are significant variations in practical methods to therapy.

Therapy options include anti-inflammatory drugs and immunosuppressants, Treatment goals include symptom control, early inflammation suppression, and end-organ damage avoidance.. To optimize efficacy while avoiding negative effects, drugs are routinely administered in combination. For optimal care, the breadth of clinical symptoms need close multidisciplinary cooperation.

Oral ulceration is typically treated with the topical use of corticosteroids, using creams or mouthwashes (5 mg prednisolone in 20 ml water, four times daily (Kontogiannis et al.,2000), or a corticosteroid inhaler. Topical corticosteroid therapy is beneficial for genital ulcers, although extended use may cause skin atrophy. Sucralfate topical suspension treats aphthous ulcers (Alpsoy et al.,1999).

1.2. Protein Phosphorylation

Protein phosphorylation was first discovered in the 1950. This event involves the transfer of a G phosphate from the phosphate donor ATP to the acceptor protein (Klumpp and Krieglstein, 2002). In mammalians serine, threonine and tyrosine residues are phosphate acceptor residues (Hanks et al., 1988).

Protein kinases drive phosphotransfer reactions. The catalytic subunits contain a binding site for the phosphate donor ATP and a groove in concert with the phosphate acceptor site of the protein substrate. These interactions ensure that the g-phosphate of ATP is in the appropriate position relative to the phosphoacceptor residue and the phosphotransfer reaction takes place. In addition to the catalytic subunits, many kinases contain subunits that regulate the conditions under which catalytic functions are carried out. Autoinhibitory elements that keep the kinase in an inactive conformation are one of them. Functional modules that interact with lipid factors, secondary messengers, or proteins are also among these subunits. Such elements control the kinase, limiting its function. The acceptor protein, which gains a load through phosphorylation, undergoes functional modification. As a result, the protein can become active or inactive. It can assemble protein components to form a functional complex or separate from protein

complexes. It can recognize, bind, activate or deactivate, phosphorylate or dephosphorylate an activated protein substrate (Klumpp and Krieglstein, 2002; Woodget, 1993).

1.2.1. Regulation of Protein Phosphorylation

Protein phosphorylation is regulated by a 3-layer system that can add (write) or recognize (reader) or remove (eraser) phosphate in cellular signaling proteins via phosphorylation (Figure 1.7).

There are enzymes specific to serine, threonine and tyrosine residues. Binary-specific enzymes that can phosphorylate and recognize both have also been identified (Camps et al., 2000).

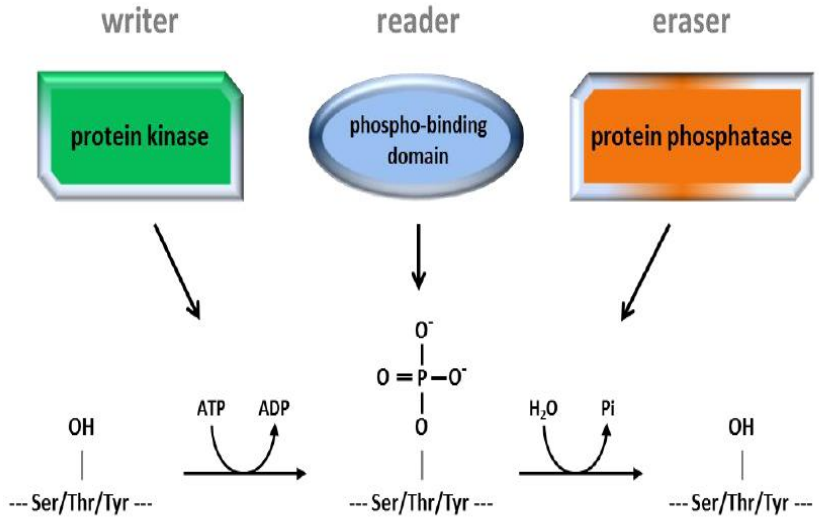


Figure 1.7. Protein phosphorylation (Jin and Pawson, 2012)

As a result of human genome sequencing studies, 518 protein kinase enzymes were identified that constitute the "authors". By catalyzing the phosphorylation reaction, these identified enzymes regulate many important

cellular processes (metabolism, transcription, cell-cycle progression, apoptosis, cell motility, cytoskeletal reorganization etc) (Manning and Cantley, 2002; Alonso et al., 2004; Moorhead et al., 2009).

Signaling molecules are molecules that have enzymatic activity and are modularly formed from regions that determine molecular interactions with nucleic acids or other proteins (Jin and Pawson, 2012).

Studies have shown that different areas have evolved to bind to specific proteins, phospholipids and nucleic acids such as SRC-homology-2 (SH2) and phosphotyrosine binding domains (PDB) (Jin and Pawson, 2012). One of many features, like proteins, PTMs, and specific peptide sequences, are targeted through domain recognition and homo/heterotypical domain-domain interactions (Grossmann et al., 2015). In addition, 14-3-3 domains and WW domains bind to the polyproline peptide that recognizes phosphorylated serine, threonine and SH3 domains, the PDZ domains on the other hand bind to other PDZ domains with specific peptide sequences at the protein carboxyl terminals (Pawson and Nash, 2003).

1.3. Protein Kinases

Protein kinases are enzymes that biochemically alter proteins as a result of the addition of a phosphate group are known as protein kinases. It is known that protein kinases, which play a central role in the formation of cellular responses such as cell proliferation and death, and the stages of intercellular signal transmission, contain approximately 500 genes in the human genome and constitute 2% of all eukaryotic genes (Manning and Cantley, 2002) (Figure 1.8).

Signal transmission is the biochemical communication between cells, and when the protein kinases responsible for this communication receive the appropriate signal, they cause changes in their enzyme activities by adding phosphate groups to histidine, tyrosine, trionine and serine amino acids found in other proteins. The addition of the phosphate group can sometimes cause about

30% of the enzymes involved in protein synthesis to be affected. Most of the events occurring in the cell are regulated by phosphorylation. Phosphorylated proteins are known to be effective in cell stabilization or degradation. At the same time, as a result of phosphorylation, proteins or fats are phosphorylated in the presence of ATP and kinases, especially in the signal transmission process, which is the most important component of intercellular communication. Proteins that are phosphorylated and thus changed in structure can thus bind to another protein or be transported to new sites such as the nucleus or mitochondria.

Considering the substrate properties, protein kinases are divided into two groups as Ser/Thr and Tyr. There are dual specific protein kinases that can phosphorylate “hem” hydroxyl groups of Ser/Thr and Try residues (Bogoyevitch and Fairlie, 2007). Ser/Thr kinases are approximately four times more abundant than Tyr kinases. Also, Ser/Thr kinases are involved in key signaling mechanisms (Arena et al., 2005).

Although cAMP-dependent protein kinase (PCA) is the secondly discovered protein kinase group, it is important because it is the first chain protein kinase, Subsequently, the transforming protein plan Src from the rous sarcoma virus was found to be associated with the catalytic chain of PCA (Barker and Dayhoff, 1982).

Analyzes of the PCA and Src sequences have shown that both share a common precursor, whereas conserved subdomains, each with a unique motif, have over time been accepted as a description of the protein kinase core (Hanks et al., 1988).

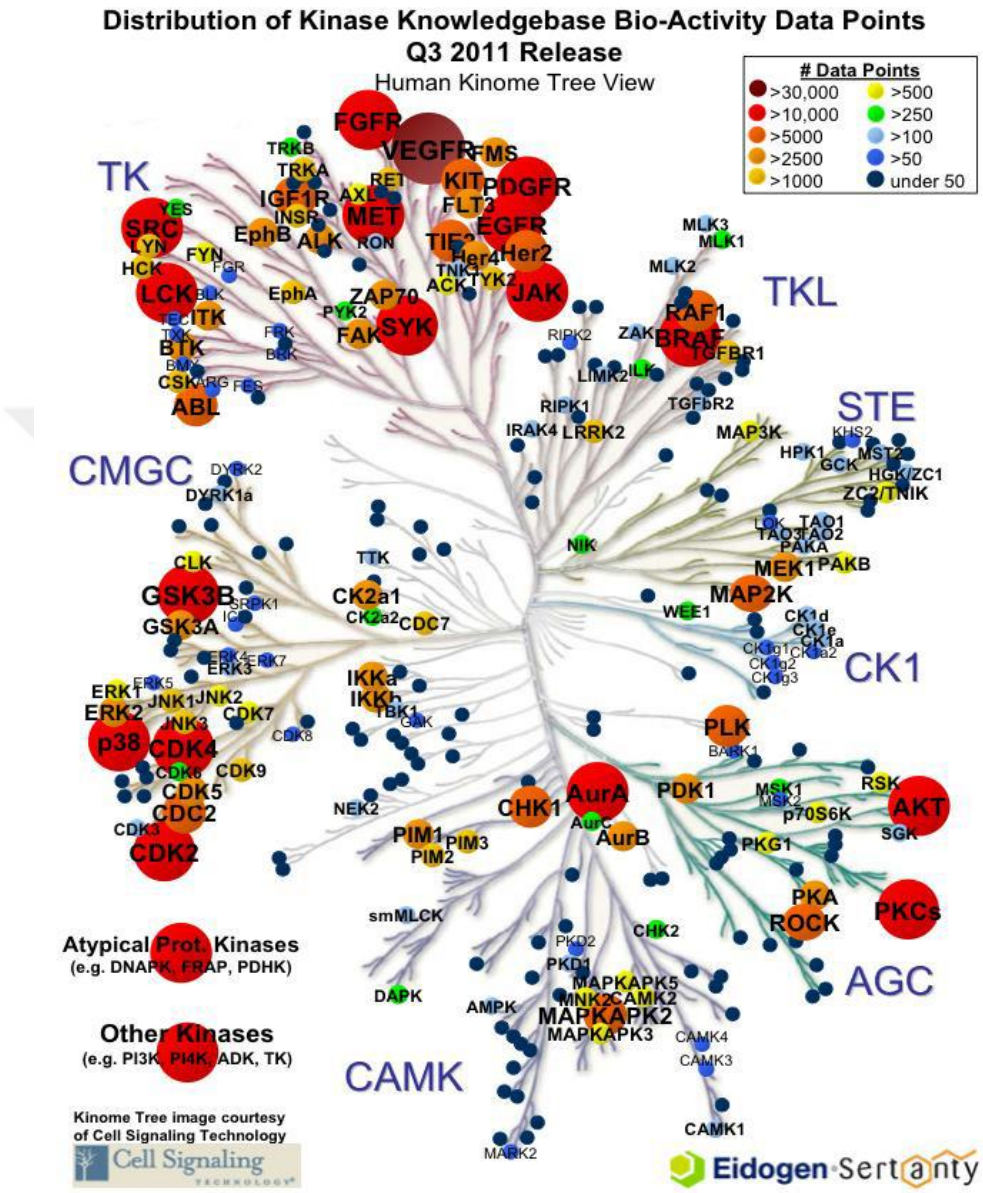


Figure 1.8. The human kinome tree

Groups are **AGC** Containing PKA, PKG, PKC families; **CAMK** Calcium/calmodulin-dependent protein kinase; **CK1** Casein kinase 1; **CMGC** Containing CDK, MAPK, GSK3, CLK families; **STE** Homologs of yeast Sterile 7, Sterile 11, Sterile 20 kinases; **TK** Tyrosine kinase; **TKL** Tyrosine kinase-like. The Kinase Knowledgebase is Eidogen-Sertanty's database of biological activity data, structure-activity relationships (SAR), and chemical synthesis data focused on protein kinases. (www.eidogen-sertanty.com)

The conserved catalytic core of protein kinases consists of two lobes, the N-terminal and the C-terminal domain (Figure 1.9). Protein kinases can be characterized as molecular switches that regulate many cellular processes (Kornev et al., 2006).

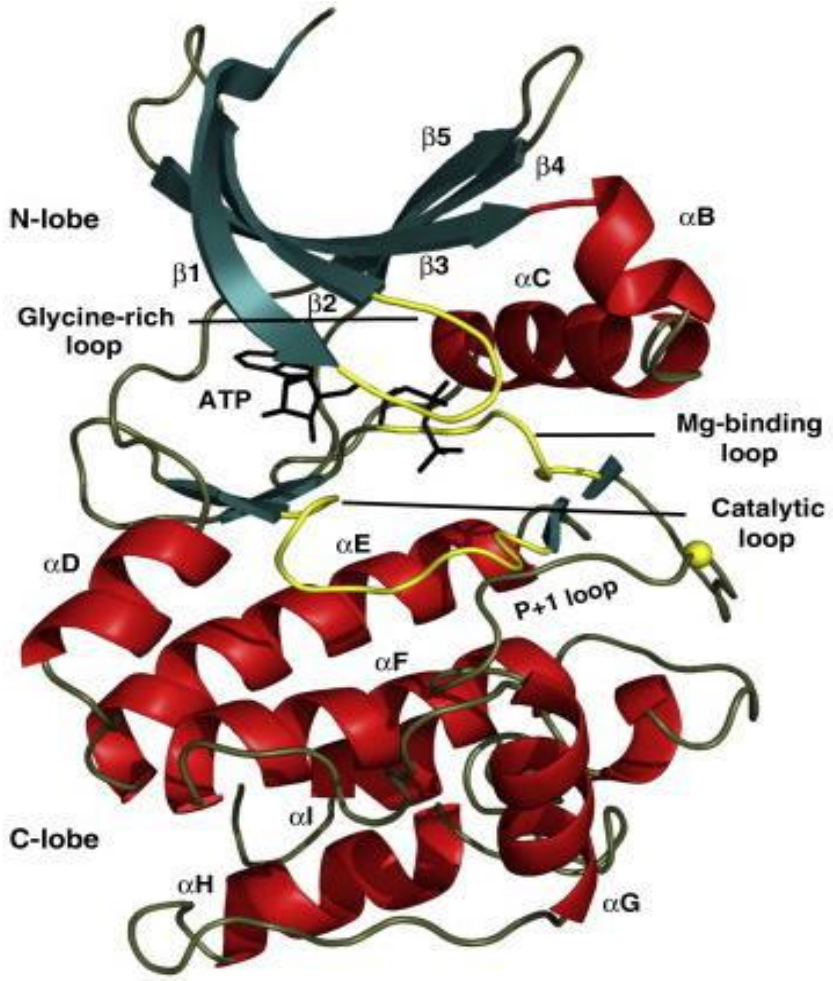


Figure 1.9. Structure of the conserved protein kinase core (Taylor and Kornev, 2011).

(PK) are composed of two domains. The N-terminal domain has Glycine-rich loop, 5 β strands (green) and the α C-helix. C-terminal domain is generally helical (red). Catalytically important loops are colored yellow. Glycine-rich loop coordinates the phosphates. The P-loop accommodates the residues of their peptide substrate (Taylor and Kornev, 2011).

1.3.1. Protein Kinase Inhibitors

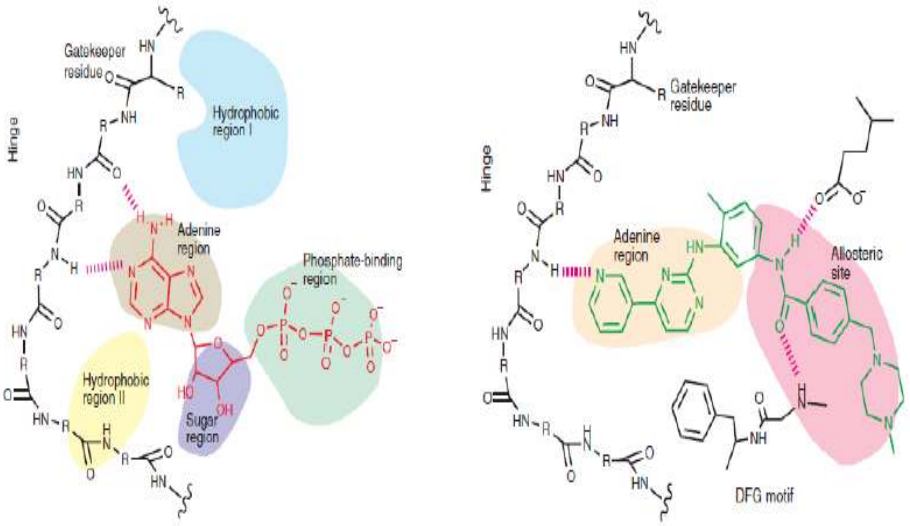
Tyrosine kinase enzymes control growth and proliferation functions in cancerous cells and lead to the growth of cancer. Silencing these molecules causes cancer cells to lose their activity. Tyrosine kinases are enzymes belonging to the protein kinase family that provide protein phosphorylation. Demonstrating the role of the increase in the functions of yrosine kinases in the pathogenesis of cancer made us think that the disease can be treated when these functions are suppressed in some way, and studies have been intensified in this direction. The first tyrosine kinase inhibitor agent whose efficacy has been demonstrated as a result of these studies is imatinib mesylate. Today, many tyrosine kinase inhibitors are used in various cancers. Agents such as erlotinib, gefitinib, afatinib used in the treatment of non-small cell lung cancer are examples of EGFR tyrosine kinase inhibitors or drugs such as crizotinib and alectinib are examples of ALK tyrosine kinase inhibitors. Regorafenib is the only tyrosine kinase inhibitor used in metastatic colon cancer. There are also several newly approved tyrosine kinase inhibitors used in lymphomas, thyroid cancers, melanoma, and rare mutations (Manning and Cantley, 2002).

Tyrosine kinase inhibitors, which are cell surface receptors for growth factors and other extracellular ligands, are effective in targeted therapy in different malignancies (Hartmann et al., 2009; Pawson, 2002, Zhang et al., 2009). The advantage of TKI is that it inhibits more than one receptor and therefore increases the probability of signal generation. Another advantage is that these compounds are used orally, so they offer ease of use to patients (Das and Wakelee, 2012). The increase in the activity of protein kinases revealed their role in cancer formation, and it was thought that cancer could be treated when these activities were inhibited. The first tyrosine kinase inhibitor compound synthesized as a result of these studies is imatinib mesylate and is a leading compound used in targeted therapy. It is thought that it can be used in the treatment of some diseases caused by tyrosine kinases (Yenerel, 2010).

Type I inhibitors: These are ATP-competitive targeting the DFG motifs of protein kinases (Zuccotto et al., 2010). These inhibitors have heterocyclic ring systems that can mimic the interaction of the adenine moiety and bind to the kinase by hydrogen bonds (Zhang et al., 2010). Figure 1.10 shows the area and subregions covered by type 1 inhibitors (Fabbro, 2012).

Type II inhibitors: These are inhibitors that target the DFG-modified conformation, which is thought to occupy the region with the hydrophobic structure adjacent to the ATP binding site (Zucottot et al., 2010).

Type III inhibitors: Since this group of inhibitors does not interact with the ATP binding domain, they are also called ATP-non-competitive or allosteric kinase inhibitors (Fig. 1.10). Considering that the binding site used is more specific to the kinase, it can be said that this group of kinase inhibitors has the highest degree of selectivity (Zhang et al., 2009).



A B

Figure 1.10. The binding modes of protein kinase inhibitors (Liu and Gray, 2006)
A) The subregions of the ATP binding site. H-bonds are represented by dashed lines B) Allosteric binding site.

1.4. Tyrosine Kinase

Tyrosine kinase enzymes control growth and proliferation functions in cancerous cells and lead to the growth of cancer. Silencing these molecules causes cancer cells to lose their activity. Tyrosine kinases are enzymes belonging to the protein kinase family that provide protein phosphorylation.

Certain signals are required for cell growth and division. There are a variety of growth factors, which are a sort of chemical. These include epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), platelet endothelial growth factor (PDGF), and fibroblast growth factor (FGF) (FGF). While EGF and FGF regulate cell growth, VEGF regulates blood vessel development while PDGF regulates both cell growth and blood vessel development. EGF interacts to a cell surface growth factor receptor, and tyrosine kinases commence signaling to initiate cell division. Tyrosine kinases predominantly transfer the phosphate group from ATP to the hydroxyl group of tyrosine residues (Fig. 1.11). When the growth factor attaches to the receptor, tyrosine kinase begins signaling cell division, resulting in cell division. The presence of a tyrosine kinase inhibitor prevents cell division by blocking the signal. Both receptor tyrosine kinases (RTKs) and non-receptor tyrosine kinases can be distinguished (NTKs). Non-receptor tyrosine kinases are found on the inner surface of the cytosol, nucleus, and plasma membrane (Kaya, 2017).

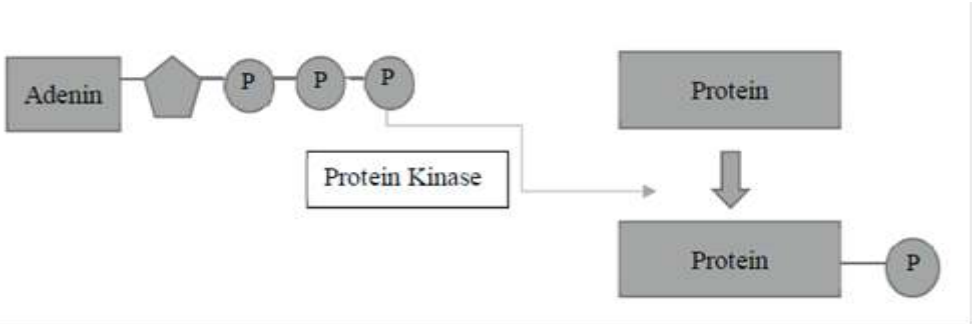


Figure 1.11. Schematic representation of the phosphorylation process by tyrosine kinase (Kaya, 2017)

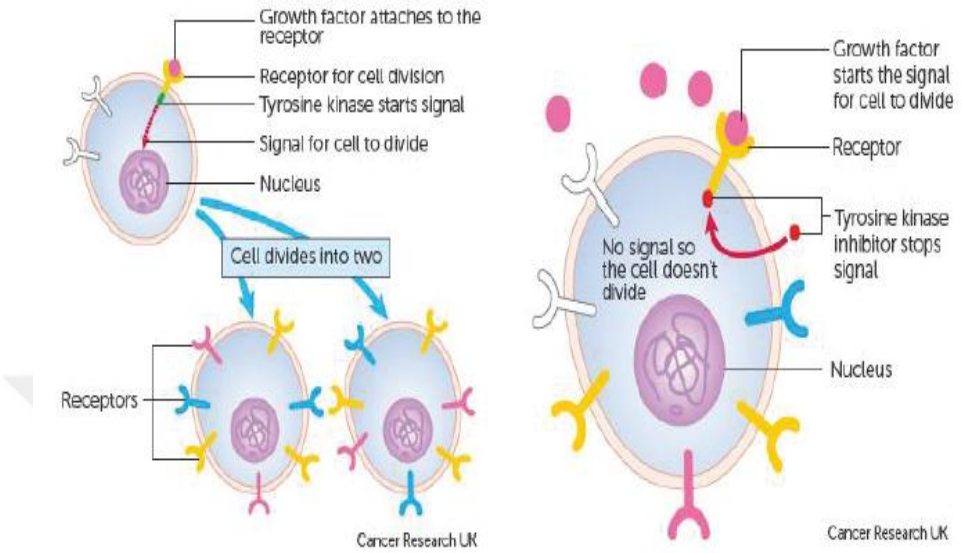


Figure 1.12. Representation of cell division control by tyrosine kinase and tyrosine kinase inhibitors (Kaya, 2017)

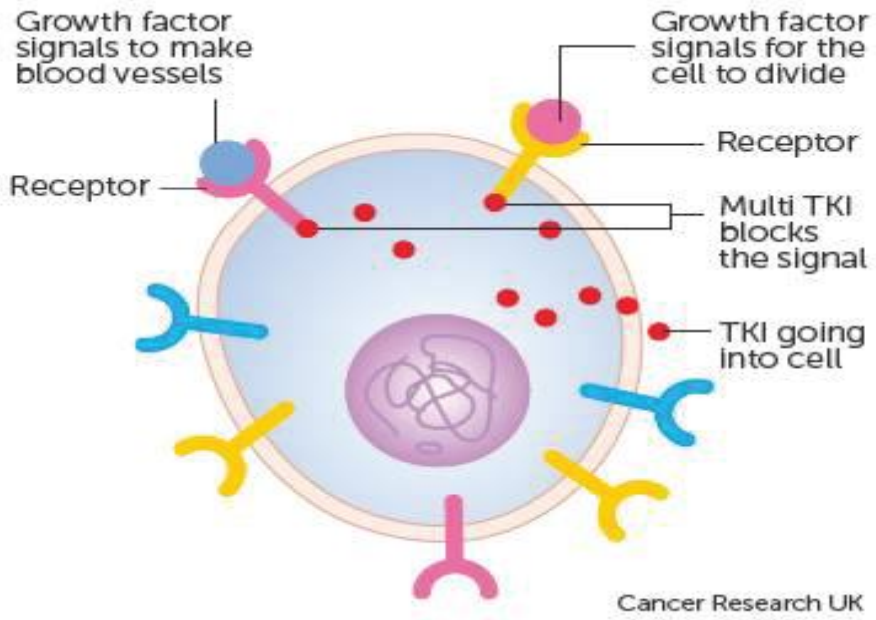


Figure 1.13. Demonstration of multiple tyrosine kinase inhibitor mechanism (Kaya, 2017)

Phosphorylation of intrinsic tyrosine residues by receptor-coupled tyrosine kinases; cytoplasmic tyrosine kinases mediate the signaling of different surface receptors (Waldburger and Firestein, 2009; Pawson, 2002). Receptor-coupled tyrosine kinases contain extracellular, while non-receptor-coupled ones contain intracellular ligand binding site (Liu and Malik, 2006).

There are approximately 60 identified receptor-coupled tyrosine kinases and they are divided into 20 subgroups (Pawson, 2002). Receptor-coupled tyrosine kinases have a distinctive structure with an immunoglobulin-like sequence, a lipophilic transmembranal segment, and intracellular carboxyl terminals with catalytic segments (Heldin, 1995; Arteaga and Johnson, 2001; Chan et al., 1992; Taniguchi et al., 1991; Sada et al., 2001).

This study was condected to investigate the Protein Tyrosine Kinases Enzyme level in Behçet's patients.

2. PREVIOUS STUDIES

Protein tyrosine kinases are important enzymes found in many regions of the signal transduction pathway that transmit cytoplasmic and extracellular information to the nucleus. Protein kinases are divided into 3 different groups such as tyrosine kinases that have onlu for tyrosine kinase residues, for serine and threonine residues and specific activity for all mentioned three residues (Bruton et al., 2009):

(1) Tyrosine kinases that are only selective to tyrosine residues in their activity
(2) Serine/threonine kinases that are selective for serine and threonine residues
(3) Kinases that are active on these three residues.

Protein kinases regulate many signal transduction pathways by catalyzing the transfer of the phosphate group of ATP to tyrosine, serine, or threonine residues in their substrate proteins, and they constitute the largest human enzyme family. As a result of protein kinase phosphorylation, the intracellular localization, stability, and substrate activity of substrates are altered.The genes of interest encode protein kinases, particularly tyrosine kinases, in many cases. TKs are enzymes that activate signal transduction pathways that regulate extracellular signal transduction, cell growth, differentiation, metabolism, migration and apoptosis. It has been shown that abnormal kinase activity is associated with many diseases, including cancer, autoimmune and inflammatory diseases (Mocsai et al., 2010; Arora and Scholar, 2005; Cheng and Force, 2010).

Protein Tyrosine kinases are among important drug targets, as they act as key mediators in the regulation of cell signaling pathways; In addition, selective and effective kinase inhibitors play a crucial part in the control of cellular activities (Mocsai et al., 2010). Abnormal activity of certain protein kinases has been detected in many human cancers, making them a molecular target for cancer

therapy. Low molecular weight TKIs such as imatinib, gefitinib, and erlotinib have been approved by the United States Food and Drug Administration (FDA), and many are in clinical trials (Bruton et al., 2009).

Studies on the relationship between protein kinase activity and various diseases are presented below.

Albin and Robinson (1980) reported that protein kinase activity increased in hepatitis B patients in their study.

Rosen et al. (1986) detected that in tumor tissues pp60c-src The activity of protein kinase rises.

Bolen et al (1987) identified an increase in pp60c-src protein kinase activity in colon cancer patients.

According to Arendt et al. (1995).'s study of Alzheimer's patients, the level of mitogen-activated protein kinase in the patient group was significantly greater than in the healthy control group.

Trembley et al. (2009) discovered that elevated protein kinase CK2 (previously casein kinase 2 or II) levels were associated with increased cell growth and proliferation in both normal and cancer cells over an extended period of time, and that CK2 also functions as an effective apoptosis suppressor. As dysregulation of cell proliferation and apoptosis are fundamental aspects of cancer cell biology, the ability to produce cancer provides a crucial link to its participation in cancer. The researchers also observed that dysregulated CK2 can disrupt both of these processes in cancer cells, with all studied malignancies displaying elevated CK2 expression, which may be associated with prognosis. Due to its influence on multiple molecular pathways that regulate cell proliferation and cell death, CK2 plays a significant role in cancer. Downregulation of CK2 by a variety of methods induces apoptosis in cultured cell and xenograft models of cancer, suggesting its therapeutic potential.

Similar to the findings of Arendt et al., (1995) found that the amount of mitogen-activated protein kinase in breast cancer patients was much higher than in

healthy individuals.As a result of the research carried out by Hosoi et al (2004) on patients with colorectal cancer, it was reported that protein kinase activity increased.

In a study by Trembley et al. (2009), High protein kinase CK2 levels are connected with cell growth and proliferation in normal and malignant tissues, and CK2 has a suppressive effect on apoptosis, according to reports.

Gloeckner et al (2009) In their investigation, they found that the disease-related protein kinase LRRK2 is elevated in Parkinson's patients.

According to the findings of a study that was carried out on Behcet's patients by Nagafuchi et al. (2005), Txk, a member of the Tec tyrosine kinase family, was overexpressed, which resulted in an excessive amount of Thl cytokine production by T lymphocytes. The research was carried out on Behcet's patients.

Mihara and Suzuki (2007) said that Txk/Rlk, a member of the Tec tyrosine kinase family, is an essential signaling mediator and that Txk expression is elevated in Behcet's disease.

Nagafuchi et al. (2005) reported in their study that overexpression of Txk, a member of the Tec tyrosine kinase family, contributes to excessive Thl cytokine production by T lymphocytes in Behçet's patients.

According to a study by Kandemir et al. (2017), in the peripheral blood mononuclear cells of Behçet's patients, LPS-induced Src family kinase activity mediated IL-10 production through STAT3 activation.

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3. MATERIAL AND METHODS

3.1. Ethics Approval of Research

Before beginning the study, consent was acquired from the Clinical Research and Ethics Committee Çukurova University.

3.2. Study Group

Applied to the Departments of Internal Medicine and Rheumatology at Çukurova University between May 2020 and May 2022. Blood samples were taken from the patients and their plasma were obtained by centrifugation at 3000 rpm for 15 minutes. until the study execution, the samples were kept at -80°C.

Two groups were created of the study: control and patient groups.

- Control Group: Healthy individuals without disease (n = 50)
- Patient Group: Behçet's patients (n = 50)

Figure 3.1. Blood samples from participants

All reagents used in the research are of analytical purity and are supplied by the MyBioSource company Cat.No: MBS014819 (USA).

3.3. Materials , Solutions and Devices Used in the Study

The materials and devices used in the research are as follows:

- Incubator (Hereaus, Germany)
- Centrifuge device (Harrier, UK)
- Automatic micropipette (10µl, 100µl, 1000µl scales, Gilson)
- ELISA Washing Device
- ELISA reader.
- ELISA Kit protein Tyrosine Kinase
- Distilled water
- Refrigerator (-80°C) (Bosch)
- Absorbent papers
- HRP Conjugate Reagent
- Wash Solution
- Stop Solution
- Chromogen Solution A
- Chromogen Solution B
- Sample Diluent
- Standards
- Microelisa Stripplate
- Closure Plate Membrane

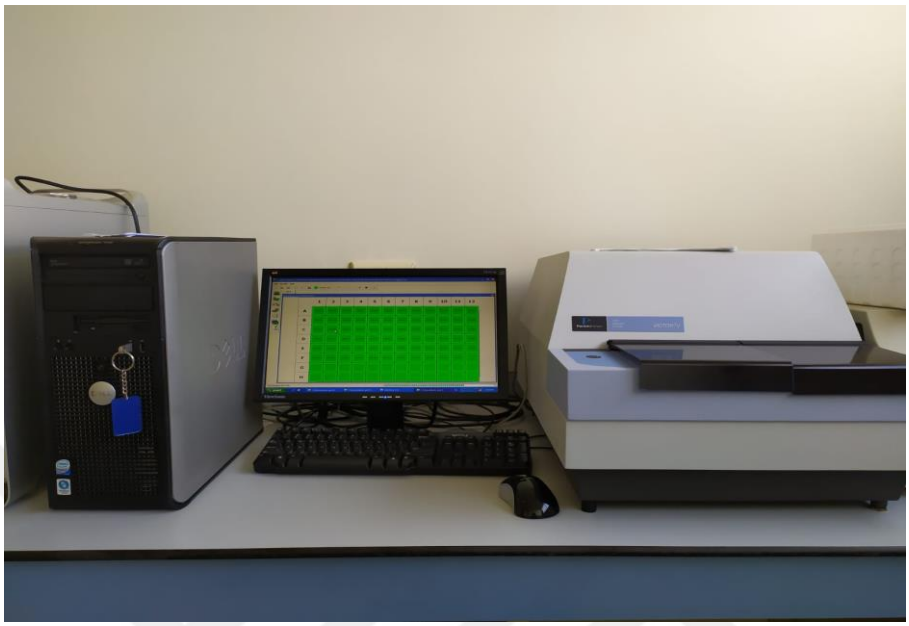


Figure 3.2. ELISA reader with 450 nm absorbance measurement capability

The ELISA method is a very fast and sensitive method used to detect and measure antigens or antibodies. In this method, the solid phase consists of polystyrene cassettes of wells for the kits. The task of the solid phase is to immobilize the antigen or antibodies in the sample. After incubation, the cassettes were washed and unbound substances were removed from the environment. It was incubated a second time after the addition of the sample and conjugate. Here the conjugate is often labeled with an enzyme, it is an antibody. Depending on the method used, the conjugate is either solid phase or bound. The cassette was washed and incubated with substrate added. As a result, enzyme and color changes were evaluated due to the reaction of the substrate in the ELISA cassette reader. Protein Tyrosine Enzyme (PTK) levels were measured according to the signal pathways determined in this study and the samples obtained.

Principle of the Assay

PTK kit was used to determine in vitro tyrosine kinase activity. The PTK kit based on the ELISA method using a multi-well plate coated with a polymer substrate specific for protein tyrosine kinase.

- Multiwell plates are pre-coated with the random polymer substrate poly-glu-tyr (PGT) containing numerous tyrosine kinase residues; PGT can be phosphorylated by several PTKs.
- The phosphorylation was begun by adding a PTK to the Mg2+, Mn2+, and ATP containing TK reaction buffer.
- Phosphorylated substrates probed with a purified phosphotyrosine-specific antibody coupled to horseradish peroxidase (HRP).
- Color augmented by HRB chromogenic substrate (OPD).
- The relative amount of tyrosine kinase activity in the sample was evaluated through spectrophotometry (ELISA reader) and quantification of the color.
- The quantitative tyrosine kinase activity in the sample was derived from the EGFR control or approximated with the use of the EGFR standard curve.

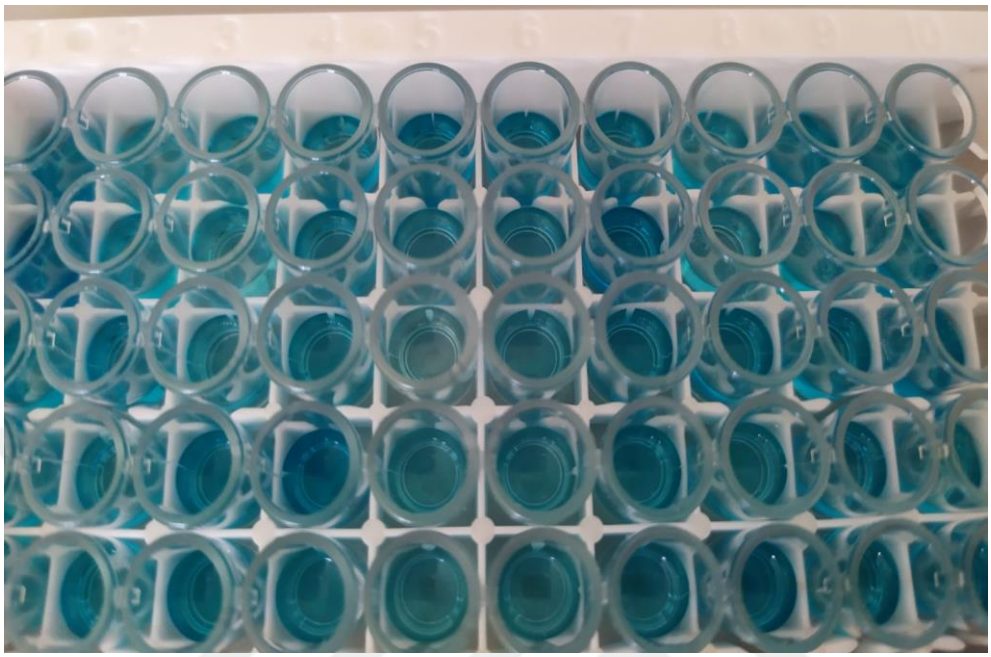


Figure 3.3. Continuous reaction in the ELISA cassette (plasma Behcet's patient)

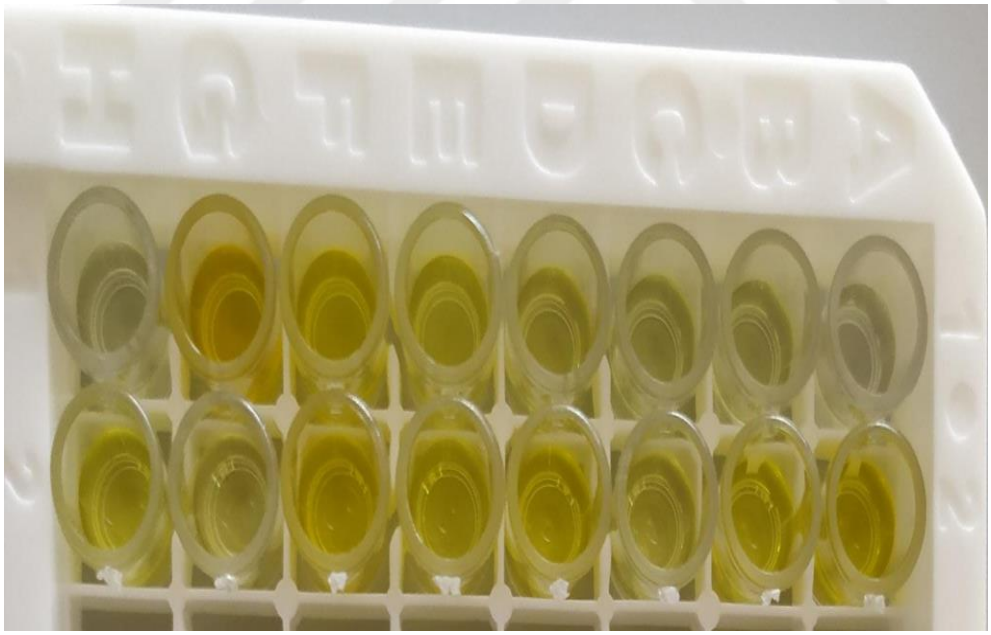


Figure 3.4. Stopping the reaction in the ELISA cassette (plasma Behcet's patient)

Figure 3.5. Mix Stop and Continuous reaction in ELISA cassette (plasma Behcet's patient)

3.3.1. Assay Procedure

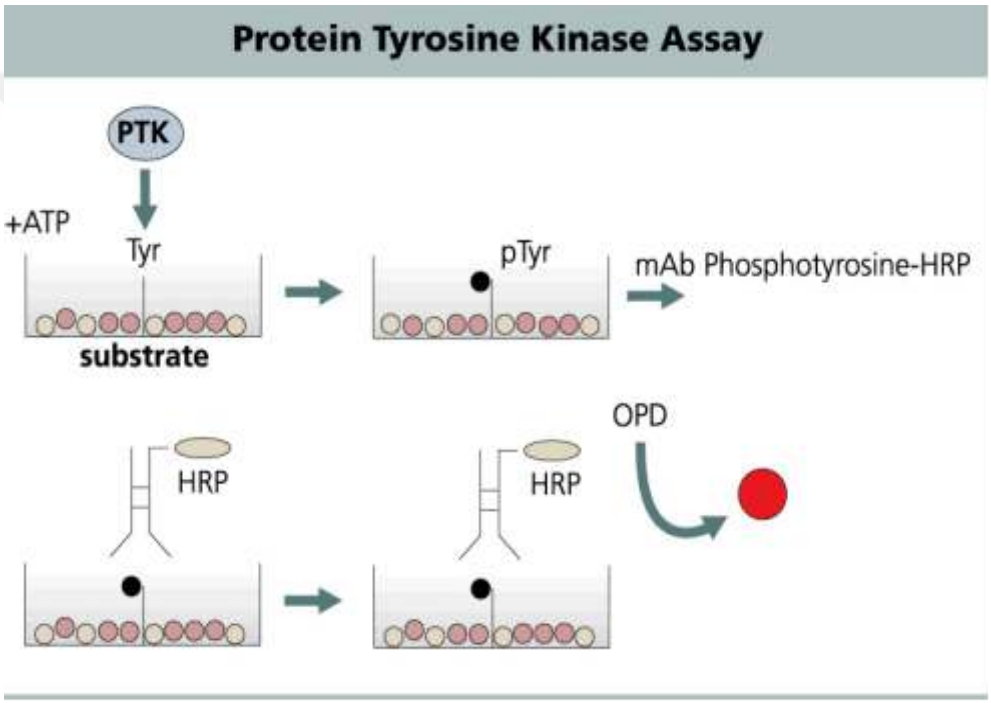
1. All substances and samples were naturally brought to room temperature (15-25°C) for 30 minutes before to processing.
2. Blank/control wells, sample wells, and standard EGFR wells were identified.
3. Each standard well was placed with a 50 µl standard.
4. Each sample well was placed with a 50 µl sample.
5. Each blank/control well was placed a 50 µl of sample diluent.
6. Duplicate of all standards, samples, and sample diluents were applied to the plate.
7. To each well were placed 100 µl of HRP-conjugate reagent.
8. After being closed, the wells were incubated for 60 minutes at 37°C.
9. There were four washings of the plate.

10. 50 µl of solution A and 50 µl of solution B were placed to each well, respectively.

11. Incubated in the dark for 15 minutes at 37 °C.

12. Placed each well with 50 µl of stop solution.

13. After adding the stop solution, optical densities at 450 nm were assessed with an ELISA reader 15 minutes later.



3.4. Statistical Analysis

For data analysis, the SPSS 21.0 program was used. As descriptive statistics, the mean and ± standard deviation were provided.

As a result of the analysis, Protein Tyrosine Kinase Enzyme level was found to be 5.57 (3.91-7.83) ng/ml in Behçet patients and 2.42 (1.26-3.63) ng/ml in healthy controls. Consequently of the independent sample T-test performed to

determine whether the Protein Tyrosine kinase levels in Behçet patients and healthy control groups differed, it was determined that Protein Tyrosine Kinases level (5.57±1.20 ng/ml) and Protein Tyrosine kinase level in Behçet patients were significantly higher than in the healthy controls (2.42±0.72 ng/ml) ($p<0.001$).

4. RESULTS AND DISCUSSION

4.1. Results

4.1.1. Descriptive statistics

The study included a total of 100 human , 50 (50%) Behçet Patients and 50 (50%) healthy (Table 4.1).

Table 4.1. Study group

		F	%	Valid %	Cumulative %
Study Group	Behcet's Patients	50	50.0	50.0	50.0
	Healthy Control	50	50.0	50.0	100.0
	Total	100	100.0	100.0	

While 33 (54.1%) of the study Behçet patients were male ,17 (43.6%) were female, 28 (45.9%) of the healthy human were male and 22 (56.4%) were female (Table 4.2).

Table 4.2. Gender distribution by groups

		Gender					
		Female		Male		Total	
		N	%	N	%	N	%
Group	Behcet's Patients	17	43.6	33	54.1	50	50
	Healthy Control	22	56.4	28	45.9	50	50
Total		39	100	61	100	100	100

While the mean gender Tyrosine kinase of Behçet patients was 5.6±1.15, when compared to the control individuals was 2.7±0.6. There was no statistically significant difference between the groups ($p>0.05$) (Table 4.3).

Table 4.3. Mean Gender by groups

	Group	N	Mean	SD (±)	T	P.value
GENDER	M.P.PTK	33	5.5	1.236	83.06 **	0.00004
	F.P.PTK	17	5.6	1.153		
	M.Con.PTK	28	2.2	0.725		
	F.Con.PTK	22	2.7	0.650		

M = Male F = Female P= Patients Con= Control Healthy PTK = Protein Tyrosine Kinase

While the mean age of Behçet patients were 5.744±1.234, when compared to the mean age of healthy individuals was 2.761±0.692. There was significant differences between two group Behçet patients and control .

Whereas the patients groups which we seen no differences between its group also the control groups the same results were obtained (Table 4.4).

Table 4.4. Mean age by groups

	Group	N	Mean	SD (±)	T	P. Value
age	P.G1.PTK	18	5.744	1.234	51.54 **	0.00002
	P.G2.PTK	14	5.101	0.999		
	P.G3.PTK	18	5.654	1.277		
	C.G1.PTK	11	2.761	0.692		
	C.G2.PTK	13	2.122	0.652		
	C.G3.PTK	26	2.457	0.724		

G1 = 30 - 40 Years G2 = 41 - 50 Years G3 = 51 > M = Male F = Female P= Patients C= Control PTK = Protein Tyrosine Kinase

4.1.2. Within-group comparisons

4.1.2.1. Findings on Protein Tyrosine Kinase Levels in Behçet's Patients

The Protein Tyrosine Kinase level of BD included in the study was between 3.91 and 7.83 ng/ml, and the mean Protein Tyrosine Kinase level was determined as 5.57±1.20 ng/ml (Table 4.5).

Table 4.5. Mean Protein Tyrosine Kinase level in BD patients

	N	Minimum	Maximum	Mean	SD (±)
Protein Tyrosine Kinase (ng/ml)	50	3.91	7.83	5.57	±1.20

4.1.2.2. Findings on Protein Tyrosine Kinase Levels in Healthy Control Group

The Protein Tyrosine Kinase level of the healthy control included in the Study was between 1.26 ng/ml and 3.63 ng/ml, and the mean protein kinase level was determined as 2.42±0.72 ng/ml (Table 4.6).

Table 4.6. Mean Protein Tyrosine Kinase level in the healthy control group

	N	Minimum	Maximum	Mean	SD (±)
Protein Tyrosine Kinase (ng/ml)	50	1.26	3.63	2.42	±0.72

4.1.2.3. Comparisons between Groups

As a result of the independent sample T-test performed to determine whether the PTK levels in BD and healthy control groups differed, it was determined that the Protein Tyrosine Kinase level in BD (5.57±1.20 ng/ml) was significantly higher than in the healthy controls (2.42±0.72 ng/ml) ($p<0.001$) (Table 4.7 ; Figure 4.1).

Table 4.7. Comparison of Protein Tyrosine Kinase levels in BD patients and healthy volunteers

	Group	N	Mean	SD (±)	T	P. Value
Protein Tyrosine Kinase (ng/ml)	Behcet Patients	50	5.57	1.20	15.563**	0.0006
	Healthy Control	50	2.42	0.72		

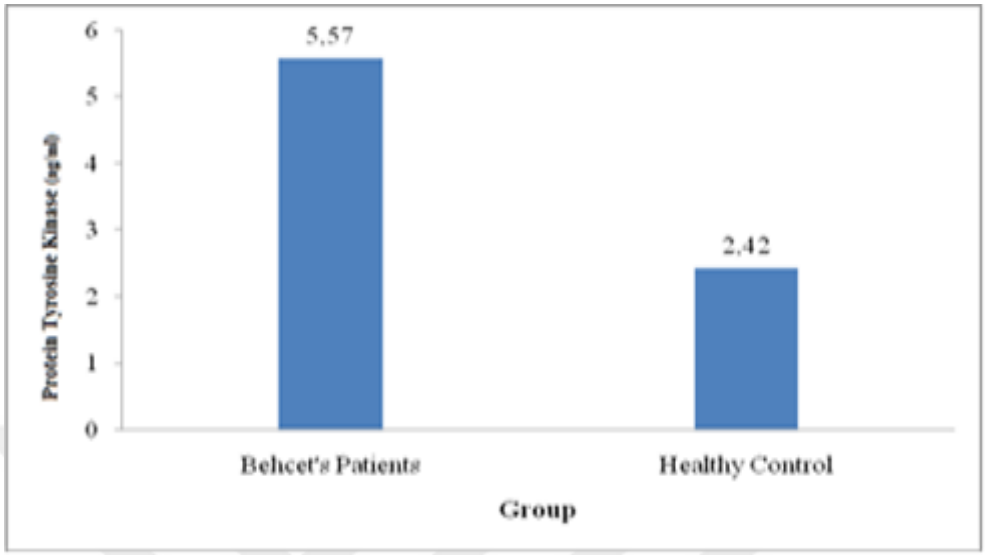


Figure 4.1. Protein Tyrosine Kinase levels by study group

4.2. Discussion

The biggest family of human enzymes, Protein Tyrosine Kinases regulate many signal transduction pathways by facilitating the transfer of the phosphate group of ATP to tyrosine, serine, or threonine residues in their substrate proteins. By phosphorylating substrates of protein kinases, intracellular localization, substrate stability, and substrate activity are altered.The genes of interest encode protein kinases, particularly tyrosine kinases, in many cases. TKs have an important role in the transmission of extracellular signals, activating signal transduction pathways that regulate cell growth, differentiation, metabolism and migration, and apoptosis (Mocsai et al., 2010). In pathological conditions such as cancer, mutated or overexpressed Kinases have a crucial function in the proliferation of proliferation and in the suppression of proapoptotic proteins, activation of anti-apoptotic factors, and/or activation of cellular pathways that lead to the spread of angiogenesis (Arora and Scholar, 2005; Cheng and Force, 2010) .

Protein kinases are among important drug targets, as they act as key mediators in the regulation of cell signaling pathways; In addition, selective and

effective kinase inhibitors play an important role in cellular function regulation (Mocsai et al., 2010). Abnormal activity of certain protein kinases has been detected in many human cancers, making them a molecular target for cancer therapy (Bruton et al., 2009).

Albin and Robinson (1980) reported that protein kinase activity increased in hepatitis B patients in their study.

Rosen et al. (1986) the study undertaken to investigate pp60c-src protein kinase activity in human tumor cell lines and tissues revealed an increase in pp60c-src protein kinase activity in tumor tissues.

Bolen et al (1987) reported that pp60c-src protein kinase activity increased in patients with colon cancer.

As a result of the study performed by Arendt et al. (1995) on Alzheimer's patients, it was reported that the level of mitogen-activated protein kinase was significantly higher in the patient group than in the healthy controls.

Mueller et al (2000) In a study of patients with primary breast cancer, the activity of mitogen-activated protein kinase was found to be a possible survival indicator.

Hosoi et al (2004) reported an increase in protein kinase activity in a study on DNA dependent protein kinase activity and upregulation of SP1 in colorectal cancer.

In a study by Trembley et al. (2009), It was found that high levels of the protein kinase CK2 are connected with cell growth and proliferation in both normal and malignant tissues, and that CK2 has a suppressive effect on the natural process of cell death known as apoptosis. Because dysregulation of cell proliferation as well as apoptosis is one of the essential characteristics of cancer cell biology, the capacity of this factor to produce cancer provides a significant link with its participation in cancer. The aforementioned researchers also found that abnormal CK2 might disrupt both of these processes in cancer cells and revealed that an increase in CK2 level was related with a bad prognosis.

Gloeckner et al (2009) reported that the disease-related protein kinase LRRK2 in their study is higher in Parkinson's sufferers.

In a study conducted on Behçet's patients by Nagafuchi et al. (2005), it was shown that Txk, a member of the Tec tyrosine kinase family, was overexpressed, causing T cells to produce an excessive amount of Thl cytokine.

Mihara and Suzuki (2007) stated in their study that Txk/Rlk, Txk, a member of the Tec tyrosine kinase family, is a key signaling mediator, and its expression is elevated in Behçet disease.

In a study by Kandemir et al (2017), In Behcet's patients, LPS-induced Src family kinase activity caused IL-10 production via activating STAT3 in peripheral blood mononuclear cells.

When the studies are examined, it is seen that the protein kinase expression level decreases with the increase in age. Ricciarelli et al. (1999) reported that there is a negative correlation between age and protein kinase expression level in their study. According to the research conducted by Boehmer et al. (2004), the expression level of protein kinase decreases with advancing age.

In our study, it was discovered that Behçet patients higher levels of the enzyme Protein Tyrosine Kinase than healthy human.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

The results obtained from the study are as follows:

1. In Behçet patients, there was no significantly different in Protein Tyrosine Kinase levels between male and female.
2. There was significant difference in Protein Tyrosine Kinase levels between Behçet patients group and control group in human age
3. Protein Tyrosine Kinase level was found to be significantly higher in Behçet patients compared to the healthy individuals.

5.2. Recommendations

In accordance with the study findings, the following recommendations were made:

1. Many enzyme parameters can be examined in both groups.
2. Studies can be repeated in different disease types.

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CURRICULUM VITAE

Akram Mudher Kareem ALJUBOURI. Finished my Primary, Middle and High school in Kirkuk city 2008. Attended to Chemistry department/ College of Scince in Kirkuk University and for four years to graduate in 2013 as a Chemist.

Also graduated from Gaziantep University, Faculty of Arts and Sciences, Biochemistry Department in 2017 as a Biochemist.

I had the apportunity to join Cukurova University in 2018 and here is the day 19 July 2022 to defend my thesis with pride.

APPENDICES

Appendix 1. Clinical Study Permissions -Turkey Republic Cukurova University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee

Appendix 2. Protein Tyrosine Kinase Enzyme values in Behçet's patients with Control healthy human .

number	gender	age	PTK BD	gender	age	PTK Control
1.	M	48	4.23	M	38	1.26
2.	M	38	5.06	M	31	3.20
3.	M	65	4.54	M	44	1.97
4.	M	35	7.43	M	58	2.65
5.	F	38	7.12	M	56	1.34
6.	M	41	4.31	M	52	1.62
7.	M	52	7.67	F	48	1.85
8.	M	51	7.83	M	36	2.21
9.	F	47	7.59	F	31	3.04
10.	F	38	7.12	M	43	1.38
11.	M	41	5.97	M	48	1.73
12.	F	36	5.53	M	40	3.59
13.	M	29	6.76	F	35	3.12
14.	F	61	4.66	M	44	1.66
15.	F	59	5.93	F	59	2.37
16.	M	55	4.58	M	63	3.34
17.	M	38	6.68	F	60	3.12
18.	M	32	6.52	M	58	2.57
19.	M	62	4.82	M	49	1.62
20.	F	54	5.93	M	64	2.76
21.	M	56	5.93	F	58	2.96
22.	F	37	7.55	M	44	2.65
23.	M	64	7.79	M	39	2.41
24.	F	58	5.30	M	65	1.66
25.	F	41	5.18	F	61	2.29
26.	M	56	5.57	F	54	3.63
27.	M	38	4.82	F	38	3.20
28.	M	54	6.76	M	59	1.42
29.	M	43	6.40	M	46	2.84
30.	M	54	4.74	M	46	1.62
31.	F	33	5.18	F	38	3.52
32.	F	42	3.91	F	62	3.24
33.	M	61	7.00	F	56	2.80
34.	M	57	4.39	F	47	3.52
35.	M	35	4.74	F	52	2.01
36.	M	48	5.12	F	40	2.37
37.	F	39	5.61	M	62	1.66
38.	M	47	4.78	M	51	1.54
39.	M	32	3.99	F	42	2.96
40.	M	58	4.04	F	65	2.13
41.	F	44	4.54	M	44	2.02
42.	M	38	6.56	M	38	2.45
43.	F	35	4.66	F	61	2.33
44.	M	38	4.03	F	45	1.77
45.	F	48	4.11	F	52	3.40
46.	M	58	4.31	M	57	3.04
47.	M	44	5.34	M	65	3.20
48.	M	47	4.86	F	61	1.42
49.	F	48	5.08	F	52	2.00
50.	M	38	4.02	M	63	3.38