

# Effect of vitamin D on Expression of microRNA-22 and microRNA-125b in Behcet Disease

*Thesis*

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# Abstract

Behcet's Disease (BD) is a chronic inflammatory disease with exacerbations and remissions characterized by recurrent orogenital ulcerations, ocular manifestations, arthritis, and vasculitis. In addition, neurological and large vessel involvement can occur. The etiology and pathogenesis of Behcet's Disease have not been clearly defined. However, several genetic, environmental, and immunological factors have been suggested as causative factors in this disease. The primary mechanism of the damage is an overactive immune system that seems to target the patient's own body. The involvement of a subset of T cells seems to be important. Vitamin D has long been known to be important for bone health and turnover. It has major biologic activities including cellular proliferation and differentiation, immune system modulation and muscle strengthening. A growing body of evidence supports the hypothesis that vitamin D is an environmental factor important in the etiology of T-cell-mediated autoimmune diseases. The biological effect of vitamin D is thought to occur by binding to its receptor (VDR) which belongs to the steroid receptor superfamily. VDR gene polymorphisms cause functional differences in immuno-modulatory action of vitamin D. This receptor is widely expressed in many cell types including antigen-presenting and lymphocytes cells. There is significant decrease in the level of Vitamin D in patient with Behcet disease.

**Conclusion:** The expression of microRNA 125b shows its statistically significant increase in patients of Behcet disease. Although the expression of microRNA 22 shows no significant difference between patients and control however its expression is lower in patients receiving steroid treatment compared to those who do not receive the treatment.

**Keywords:** vitamin D, microRNA-22, microRNA-125b, Behcet Disease

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## List of Abbreviations

<b>AD</b>	Allelic discrimination
<b>Ago</b>	Argonaute
<b>Alb</b>	Albumin
<b>ALK</b>	Alkaline phosphatase
<b>ALT</b>	Alanine transaminase
<b>ANA</b>	Antinuclear antibody
<b>ASO</b>	Anti-sense oligonucleotides
<b>AST</b>	Aspartate transaminase
<b>Bcl-Xl</b>	B-cell lymphoma-extra large
<b>BIC</b>	B-cell integration cluster
<b>Bil</b>	Bilirubin
<b>BD</b>	Behcet's Disease
<b>bp</b>	Base pair
<b>BT</b>	Breakthrough
<b>CBC</b>	Complete blood count
<b>CD</b>	Cluster of differentiation
<b>CHC</b>	Chronic hepatitis C
<b>CLDN1</b>	Claudin-1
<b>CsA</b>	Cyclosporin A
<b>CYP3A4</b>	cytochrome P450, family 3, subfamily A, polypeptide 4
<b>DAA</b>	Direct acting antiviral
<b>DGAT</b>	Diacylglycerol O-acyltransferase
<b>VDR</b>	Vitamin D Receptors
<b>DNA</b>	Deoxyribonucleic acid
<b>dNTPs</b>	Deoxy-nucleotide triphosphate
<b>miRNA</b>	Micro Ribonucleic acid
<b>ds-RNA</b>	Double stranded RNA
<b>ISG</b>	International Study group
<b>EDTA</b>	Ethylenediaminetetraacetic acid
<b>EGFR</b>	Epidermal growth factor receptor
<b>eIF3</b>	Eukaryotic initiation factor
<b>ELISA</b>	Enzyme-linked immunosorbent assay
<b>VEGF</b>	vascular endothelial growth factor

<b>ICBD</b>	International Criteria of Behcet's Disease
<b>ER</b>	Endoplasmic reticulum
<b>RAS</b>	Recurrent aphthous stomatitis
<b>EVR</b>	Early virlogical response
<b>BSAS</b>	Behcet's Syndrome Activity Scale
<b>Glu</b>	Glucose
<b>Gt</b>	Genotype
<b>GWAS</b>	Genome wide association study
<b>ESR</b>	Erythrocyte Sedimentation Rate
<b>CBC</b>	Complete Blood Count
<b>EULAR</b>	European League Against Rheumatism
<b>HCC</b>	Hepatocellular carcinoma
<b>HCV</b>	Hepatitis C virus
<b>TNF</b>	Tumor necrosis factor
<b>HTA</b>	Host targeting antiviral
<b>IgG</b>	Immunoglobulins G
<b>Igf</b>	insulin-like growth factor
<b>IFN</b>	Interferon
<b>IPS-1</b>	Interferon $\beta$ promoter stimulator
<b>IRES</b>	Internal ribosome entry site
<b>IRF</b>	Interferon regulatory factor
<b>PAAs</b>	Pulmonary artery aneurysms
<b>ISGF</b>	Interferon-stimulated gene factor
<b>IL</b>	Interleukin
<b>IMPDH</b>	Inosine monophosphate dehydrogenase
<b>JAK</b>	Janus kinase
<b>JNK</b>	c-Jun N-terminal kinase
<b>LDs</b>	Lipid droplets
<b>LNA</b>	locked nucleic acid
<b>miRNA</b>	MicroRNAs
<b>mRNA</b>	Messenger ribonucleic acid
<b>AZA</b>	Azathioprine
<b>NF-<math>\kappa</math>B</b>	Nuclear factor kappa-light-chain-enhancer of activated B cells
<b>ncRNAs</b>	Non-coding RNAs
<b>NI</b>	Nucleoside analog inhibitor

<b>NL</b>	Null response
<b>nt</b>	Nucleotide
<b>NTC</b>	Non template control
<b>OCLN</b>	Occludin
<b>ORF</b>	Open reading frame
<b>P-bodies</b>	processing bodies
<b>PBMC</b>	Peripheral blood mononuclear cells
<b>PCR</b>	Polymerase chain reaction
<b>PEG</b>	Polyethylene glycol
<b>PegIFN</b>	Pegylated interferon
<b>pH</b>	Potential hydrogen
<b>PIs</b>	Protease inhibitors
<b>PI-KA</b>	Phosphatidyl inositol kinase A
<b>Pol II</b>	Polymerase II
<b>PPAR</b>	Peroxisome proliferator-activated receptor
<b>pre-miRNA</b>	precursor miRNA
<b>pri-miRNA</b>	primary miRNA
<b>PRKRA</b>	protein kinase, interferon-inducible double- stranded RNA-dependent activator
<b>PR</b>	Partial response
<b>PT</b>	Prothrombin time
<b>Q</b>	Quencher
<b>R</b>	Reporter
<b>RBV</b>	Ribavirin
<b>RFLP</b>	Restriction fragment length polymorphism
<b>RIG-I</b>	Retenoic acid inducible gene I
<b>RISC</b>	RNA-induced silencing complex
<b>RLC</b>	RISC loading complex
<b>RNA</b>	Ribonucleic acid
<b>rpm</b>	Revolutions per minute
<b>RT-PCR</b>	Reverse transcriptase polymerase chain reaction
<b>RVR</b>	Rapid virological response
<b>S</b>	Standard
<b>SDS</b>	Sodium dodecyl sulphate
<b>SHP</b>	Src(sarcoma) homology 2-domain containing tyrosine phosphatase
<b>SL</b>	Stem loop

<b>SNP</b>	Single nucleotide polymorphism
<b>SOCS</b>	Suppressor of cytokine signaling
<b>SP</b>	Specificity protein
<b>SR-BI</b>	Scavenger receptor type B class I
<b>STAT</b>	Signal transducer and Activator of transcription
<b>SVC</b>	Spontaneous viral clearance
<b>SVR</b>	Sustained virological response
<b>T</b>	Test
<b>Taq</b>	Thermus aquaticus
<b>TGF-<math>\beta</math></b>	Transforming growth factor beta
<b>TH</b>	T helper
<b>TIR</b>	Toll/interleukin 1 receptor
<b>TLR</b>	Toll like receptor
<b>TNF</b>	Tumor necrosis factor
<b>TPV</b>	telaprevir
<b>TRIF</b>	TIR domain-containing adapter-inducing interferon $\beta$
<b>TRBP</b>	Trans-activation response RNA-binding protein
<b>TSH</b>	Thyroid stimulating hormone
<b>TU</b>	transcription unit
<b>TyK</b>	Tyrosine kinase
<b>UTRs</b>	Untranslated regions
<b>UVB</b>	Ultra violet B
<b>VLDL</b>	Very low-density lipoprotein
<b>WHO</b>	<a href="#">World Health Organization</a>



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## INTRODUCTION

Behcet's Disease (BD) is a chronic inflammatory disease with exacerbations and remissions characterized by recurrent orogenital ulcerations, ocular manifestations, arthritis, and vasculitis. In addition, neurological and large vessel involvement can occur. The etiology and pathogenesis of Behcet's Disease have not been clearly defined. However, several genetic, environmental, and immunological factors have been suggested as causative factors in this disease. The primary mechanism of the damage is an overactive immune system that seems to target the patient's own body. The involvement of a subset of T cells seems to be important.

Vitamin D has long been known to be important for bone health and turnover. It has major biologic activities including cellular proliferation and differentiation, immune system modulation and muscle strengthening. A growing body of evidence supports the hypothesis that vitamin D is an environmental factor important in the etiology of T-cell-mediated autoimmune diseases.

The biological effect of vitamin D is thought to occur by binding to its receptor (VDR) which belongs to the steroid receptor superfamily. VDR gene polymorphisms cause functional differences in immuno-modulatory action of vitamin D. This receptor is widely expressed in many cell types including antigen-presenting and lymphocytes cells.

MicroRNAs (miRNAs) are short non-coding RNAs with wide gene regulatory activity at the posttranscriptional level. MiRNAs associate with

several proteins in RNA silencing complexes that cause mRNA degradation or translation inhibition, or both processes.

In recent years, miRNAs have been shown to play key roles in cancer as they control the expression of crucial oncogenes and tumour suppressor genes and, accordingly, several miRNAs are either over-expressed or silenced affecting many diseases.

MiRNA-22 augments tumour suppressor activity. 1,25(OH)<sub>2</sub>D<sub>3</sub> modulates cell proliferation: it usually has a mild to medium cell-type-dependent inhibitory effect, although stimulatory effects have also been reported. miR-22 is induced by 1,25(OH)<sub>2</sub>D<sub>3</sub> and contributes to its inhibitory effects on the proliferation and migration of cells.

Moreover, anti-miR-22 expression abrogates the regulation by 1,25(OH)<sub>2</sub>D<sub>3</sub> of the RNA levels of several target genes. Importantly, miR-22 is downregulated in a high proportion of colon tumours and its expression correlates directly with that of VDR. Together, miR-22 is a target of 1,25(OH)<sub>2</sub>D<sub>3</sub> and mediates in part its protective action against many diseases.

The microRNA miR-125b is multi-faceted, with the ability to function as a tumor suppressor or an oncogene, depending on the cellular context. To date, the pro-apoptotic role of miR-125b and its underlying mechanisms are unexplored. miR-125b level was positively associated with the rate of apoptosis in HCC tissues.

## AIM OF THE WORK

The aim of our study is to investigate serum levels of vitamin D and its effect on miRNA22 and miRNA125B gene expression in Egyptian patients with BD and to evaluate their relationship to disease activity.