Vitamin D metabolic genes in Behçet's Syndrome

William Ogunkolade[1], Amal Senussi[1,2], Solmaz Eghbali[1], Rahaf Alshahrani[1], Sarah Sacoor[1,2], Azimoon Bibi[1,2], Nardos Wakjira[2], Bindi Gokani[2], Yashoda Jagatiya[2], Farida Fortune[1,2]

1;-Barts and The London School of Medicine and Dentistry, Centre Immunobiology and Regenerative Medicine, Queen Mary University of London, UK
2;-London Behçet's Centre, Royal London Hospital, Barts Health London, UK

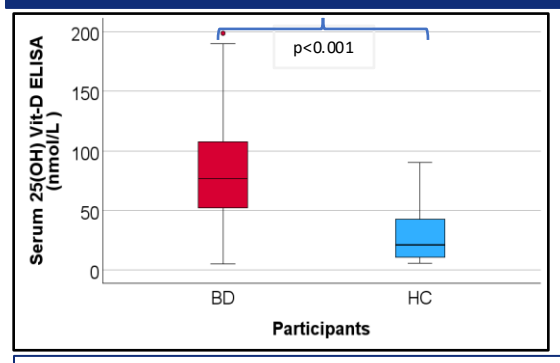
Introduction

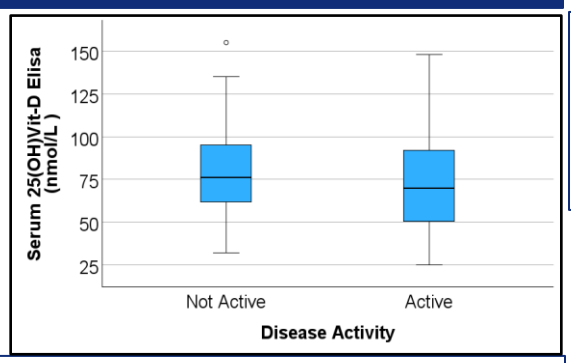
- Metabolism of cholecalciferol (VD3) and ergocalciferol (VD2), consists of several sequential enzymatic hydroxylation conversions.
- Microsomal CYP2R1 and mitochondrial CYP27A1 are the most possible enzymes responsible for vitamin D 25-hydroxylation activity.
- 1α-hydroxylase (CYP27B1), mainly in the kidney but also in extra-renal tissues, converts 25(OH)D3 to active form 1.25-dihydroxyvitamin D (calcitriol). Vitamin D receptor (VDR) mediate the action $1,25(OH)_2D_3$. The 24-hydroxylase (CYP24A1) convert both 25(OH)D3 and 1,25(OH)2D3 less active compounds 24,25(OH)2D3. All these genes are expressed all target tissues suggesting extra-hepatic production of 25(OH)VitD as well as extra-renal production of 1,25(OH)2D3 with paracrine/autocrine and a role for local bioactivation of Vit-D by CYP27A1, CYP2R1 and CYP27B1.
- In this study we investigated the relationship between serum levels of vitamin D and the relative levels of Vitamin D metabolic genes in Behçet's Syndrome (BS) patients compared to healthy controls.

Methods

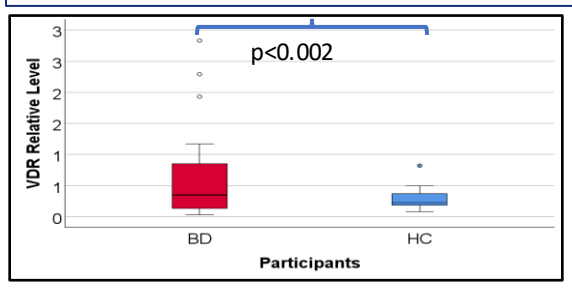
- We recruited 78 BD patients and compared them to 30 healthy controls recruited from the Blizard Institute and the Behçet's Centre of Excellence at the Royal London Hospital. BS patients were diagnosed according to the International Study Group (ISG) criteria or the International Criteria for Behçet's Disease (ICBD)
- Total serum 25-hydroxyvitamin D (25(OH)D) level was measured by using competitive immunoassays.
- Total cellular RNA was extracted using RNeasy RNA isolation kit from Ficoll-Hypaque isolated PBMCs according to the manufacturer's recommendations. cDNA was synthesized and qPCR performed with gene specific primers on **StepOne** Real-Time PCR Systems. Relative expression level was calculated relative to control sample calibrator using the Livak 2DDCT Method ($2^{(-\Delta\Delta CT)}$) method. Statistical analysis performed using SPSS version 29.

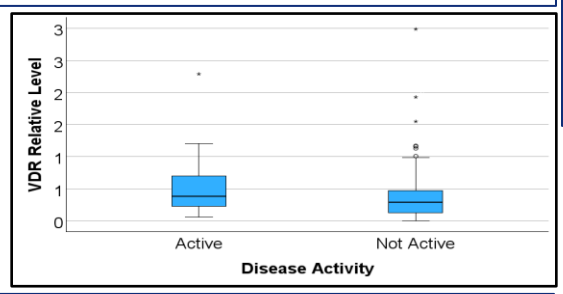
Results





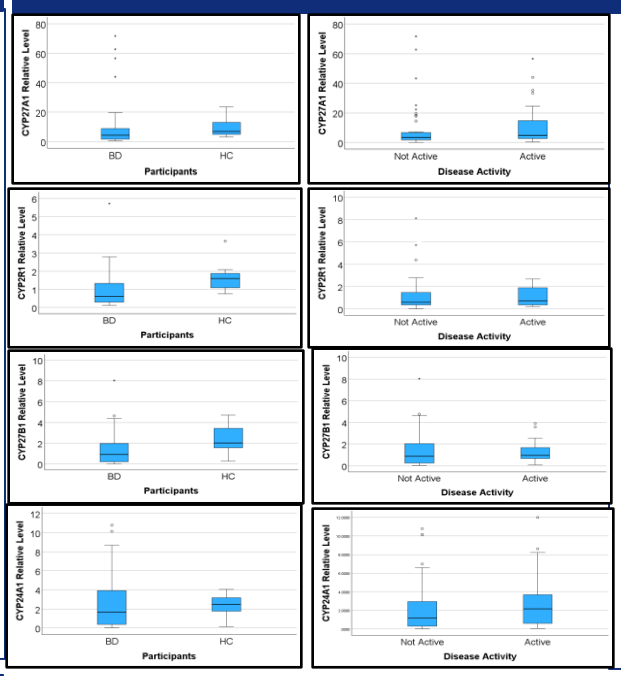
- BS patients median 25(OH)D serum concentrations (84.70 nmol/L) is higher than in healthy controls (26.418 nmol/L, p<0.001)(figure 1).
- No significant difference in median 25(OH)D levels among patients with active BS as compared to those with inactive disease.



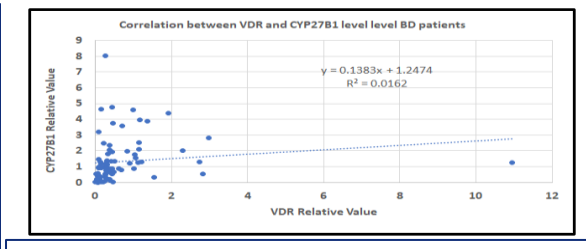


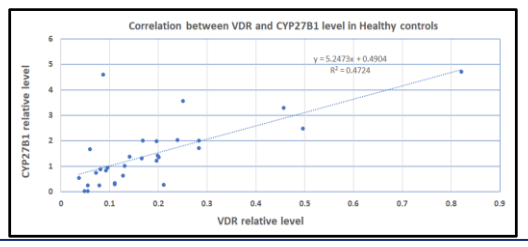
- VDR expression level is higher in BS patients than in our controls (0.6726 +/- 0.14657 vrs 0.1851 +/- 0.02982 (t= 3.26, p= 0.002).
- No significant difference in expression levels among patients with active BS as compared to those with inactive disease.

Results



- The expression levels of Vitamin D metabolic enzymes were not significantly different based on patients' gender, BS activity, and phenotype.
- Multiple regression analysis of expression data from BS patients ($F(4,77)=30.218$, $p<<0.001$, $R^2=.59$)) and healthy control ($F(4,25)=6.272$, $p<0.001$, $R^2=.50$)) suggest that Cyp27A1, Cyp2R1, Cyp27B1 and Cyp24A1 level taken together have a significant impact on VDR level.
- Cyp27B1 is the unique contributor to VDR level with a positive effect in controls and a negative effect in BS patients.





- CYP27B1 level correlate well with VDR expression in healthy controls but not in BS patients.

Coefficients[a]	Unstandardized Coefficients		Standardized Coefficients		
	B	Std. Error	Beta	t	Sig.
(Constant)	0.053	0.05		1.076	0.292
Cyp27A1 level	0.001	0.009	0.03	0.103	0.919
Cyp2R1_level	-0.045	0.057	-0.219	-0.788	0.438
Cyp27B1 level	**0.099**	**0.036**	**0.754**	**2.73**	**0.011**
CYP24A1 level	0.016	0.027	0.113	0.597	0.556
a. Dependent Variable: VDR_Relative_level					

Multiple regression Coefficients table showing unique contributor to VDR level in healthy control is CYP27B1 (B=0.099, p=0.036) and not Cyp27A1 (B=0.001, p=0.919) Cyp2R1(B=-0.045, p=0.438) or CYP24A1(B=0.016, p=0.597)

Coefficients[a]	Unstandardized Coefficients		Standardized Coefficients		
	B	Std. Error	Beta	t	Sig.
(Constant)	-0.052	0.138		-0.379	0.706
Cyp27A1 level	**0.06**	**0.01**	**0.689**	**5.925**	**<0.000**
Cyp2R1 level	0.179	0.115	0.194	1.558	0.123
Cyp27B1 level	**-0.106**	**0.036**	**-0.356**	**-2.975**	**0.004**
CYP24A1 level	0.031	0.041	0.086	0.766	0.446
a. Dependent Variable: VDR_Relative_level					

Multiple regression Coefficients table in BS showing positive influence of Cyp27A1 level (B=0.060, p<<0.0001) and negative influence of CYP27B1 level on VDR level while Cyp2R1level (B=-0.179, p=0.123) or CYP24A1 (B=0.031, p=0.446) does not affect VDR level

Discussion

- Loss of correlation of VDR with both CYP27B1 and CYP24A1 suggests a loss of VDR sensitivity to the active Vitamin D made by CYP27B1 in BS patients.
- This loss of sensitivity is further confirmed by regression analysis showing a positive influence of CYP27B1 on VDR level in healthy controls and a negative influence in BS patients despite a high level of VDR expression.
- Our data suggests that local Vitamin D metabolism in PBMC is deregulated in BS patients.