SERUM VITAMIN D AND T REGULATORY CELLS IN A GROUP OF ADULTS WITH BRONCHIAL ASTHMA

Thesis

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بسم الله الرحمن الرحيم
وأنزل الله عليك
الكتاب والحكمة
وعلّمك ما لَمْ تكُن
تَعْلَمُ وكَانَ فَضْلُ
اللَّهِ عَلَيْكَ عَظِيماً
صدق الله العظيم
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Lamiaa Salah Eldin

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List of Abbreviations

AHR	: Airway hyper responsiveness
AB	: Anti- VDBP antibody
ADAM	: Adisintegrin and metalloproteinase
APCS	: Antigen presenting cells
ASYBUF	: Assay Buffer
B_{10} cells	: regulatory B cells
HBD-2	: Human b- defensins-2
CBC	: Complete blood picture
CCL21	: CC-chemokine Ligand 21
CCR7	: CC-chemokine receptor 7
COPD	: Chronic obstructive pulmonary disease
CR2	: Complement receptor 2
CSIF	: cytokine synthesis inhibitory factor
CTL	: cytotoxic T- Lymphocytes
CTLA-4	: cytotoxic T- lymphocyte antigen 4
DCs	: Dendritic cells
DEX	: Dexamethasone
DN	: Double negative
DP	: Double positive
DRIs	: Dietary reference intakes
EAE	: Experimental autoimmune encephalomyelitis
Ebi3	: Epstein- Barr-Virus – induced gene3
EDTA	: Ethylene diamin tetra- acetate
ELISA	: Enzyme linked immune sorboent assays
FEIAS	: Fluorescent enzyme immunoassays
FEV1	: Forced expiratory volume in 1 second
FITC	: fluorescein isothio cyonate
Foxp3	: Forkhead box p3
FR	:folate receptor
FVC	: forced vital capacity
GITR	: glucocorticoid- induced TNF receptor
GITRL	: GITR ligand
GSDMB	: Gasdermin B
HEVS	: High endothelial venules
HLA	: Human leukocyte antigen
i tregs	: Inducible tres
IBD	: Inflammatory bowel diseese
ICOS	: inducible costimulatory molecules
ICS	: Inhaled Corticosteroids
IDO	: indoleamine 2,3 dioxygenase
iGb3	: iso globotrihexosylcer amide

IL : interleukin
ILt3 : immunoglobulin like transcript 3
IPEX : Immunodysregulation, polyendocrinopalhy and Enteropathy X-linked
ITAM : immunoreceptor tyrosine- based activation motif
IUKT : Induced natural killer T cells
LABA : Long- acting beta2 agonist
LAP : Latency-associated peptide
LAG3 : lymphocyte – activation gene3
LT : Leucotriens
MHC : Major histocompatibility complex
MMP : Matrix metalloproteinases
MS : Multiple sclerosis
MyD88 : Myeloid differentiation factor 88
n tregs : Natural Tregs
NFAT—API : nuclear factor of activated T cells-activation proten 1-
NKT : Natural killer T cells
NOD2 : Nucleotide – binding oligomerization domain- containing protein 2
NSB : Non specific binding control
ORMDL3 : Orosomucoid-Like3
PAMPs : Pathogen associated molecular patterns
PBS : phosphate buffer saline
PDC : Plasmacytoid dendritic cells
PD-L1 : programmed cell dealth ligand 1
PE : phycoerythrin
Pe-cy5 : Pyycoerythrin-cyanin 5
PEF : Peak expiratory flow
PG : Prostaglandins
PGI2 : Prostacyclin
RA : Rheumatoid arthritis
RANTES : Regulated upon Activation, Normal T- cell Expressed and Secreted
RASTS : Radio allergo sorbent assays
RDA : Recommended Dietary Allowance
ROG : Receptor of GATA-3
RXR :Retinoic X receptor
SCIT : subcutaneous immunotherapy
SD : Standard deviation
SDF : Stromal cell- drived factor
SIT : Allergen- specifrc inmunotherapy
SLE : systemic lupus erythematosus
SLIT : Sublingual immunotherapy
SNP : Single nucleotide polymorphism
SPF : Sun protection factor

SSPS : Statistical package for social science
STAT : Signal transducer activator of transcription
TCR : T -cell receptor
Teff : T-effector cells
TG F-β : Transforming growth factor- β
TH : T- helper cells
Th3 : T helper 3
TLR : toll-like receptors
TMB : Tetramethylbenzidine
TNF : Tumor necrosis factor
TR1 : T regulatory 1 cells
TREGS : T regulatory cells
TSLP : Thymus stimulating lipoproteins
TXA2 : Thromboxane A2
UL : Upper intake level
UVB : Ultraviolet B
VDBP : Vitamin D binding protein
VDR : vitamin D receptor
VEGF : Vascular endothelial growth factor
VSMC : Vascular smooth muscle cells
WASBUF :Wash Buffer
WBC : White Blood cell
$\overline{x}$: Mean
α –galcer : α – galactosylceramide
1,25 (OH)2D 1,25- dihydroxy vitamin D
25 (OH)D : 25- hydroxy vitamin D

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INTRODUCTION

Bronchial asthma is a chronic disorder of the airways that is characterized by reversible airflow obstruction, airway inflammation, persistent airway hyperreactivity and airway remodeling ***(Buc et al., 2009).***

The etiology of asthma is complex and multifactorial. Although profound insights have been made into the pathophysiology of asthma, the exact mechanism inducing and regulating the disease is not fully understood. According to Woodruff *et al*., asthma can be divided into at least two distinct molecular phenotypes defined by the degree of Th2 inflammation into intrinsic and extrinsic asthma **(*Woodruff et al., 2009).***

The prevalence of asthma has increased significantly since the 1970s, to reach up to 300 million worldwide affected people in 2010. In 2009, asthma caused 250,000 deaths globally. Despite this, with proper control of asthma with step down therapy, prognosis is generally good ***(Lemanske & Busse, 2010).***

Naturally occurring CD4+CD25+ Tregs (nTregs) are thymic-derived. A second population of CD4+CD25+ Tregs can be induced in vitro and in vivo through antigen stimulation. Both subsets appear to regulate immune responses through the

production of IL-10 and transforming growth factor (TGF)-beta ***(Hawrylowicz & O'Garra , 2005 ; Buckner 2010)***. These cells have the potential to protect against the development of allergic disease and asthma, in particular ***(Fontenot et al., 2003; Akdis et al., 2005).***

The discovery that many cells express vitamin D receptors and the recognition of widespread vitamin D insufficiency has stimulated interest in the potentially beneficial effects of vitamin D in nonskeletal conditions. The disorders in which vitamin D may play a role include cancer, infection, cardiovascular disease, schizophrenia, and immune-mediated diseases such as multiple sclerosis, insulin-dependent diabetes, and asthma ***(Holick, 2007).***

Several laboratories have described the capacity of vitamin D to promote regulatory T cells (Tregs). Tregs play an important role in the control of the immune response and inhibit Th2 responses, as well as airway inflammation and airway hyper-responsiveness, which may be the key to the potential role of vitamin D in asthma. Vitamin D also acts directly on T cells to promote an IL-10-secreting Treg phenotype either alone or in concert with glucocorticoids ***(Urry* et al., 2009).**

Aim of the work

The aim of this study is to determine the frequency of T regulatory cells as well as serum 25OH vitamin D levels in patients with atopic asthma and to determine whether there is a correlation between both parameters and their effect on clinical impact of the disease.

Chapter I
BRONCHIAL ASTHMA

Introduction:

Asthma is a chronic inflammatory disease of the airways associated with airway hyper responsiveness (AHR), coupled with wheezing, breathlessness, chest tightening and coughing. Characteristically, the obstruction of the airways is reversible, either spontaneously or with treatment ***(Barrett and Austen, 2009).***

There is a strong genetic association with atopy, the predisposition to produce IgE antibodies to environmental allergens. Major cellular components driving asthmatic reactions include mast cells, eosinophils, and T cells, with a prominent role for CD4+ Th2 cells. More recently, roles for basophils, induced natural killer T cells (iNKT cells), Th17 cells, and a number of soluble mediators, including thymus stimulating lipoproteins (TSLP), IL-25 and IL-33, have also been proposed ***(Barrett and Austen, 2009).***

Although asthma is a chronic obstructive condition, it is not considered as a part of chronic obstructive pulmonary disease as this term refers specifically to combinations of disease that are irreversible such as bronchiectasis, chronic bronchitis, and emphysema ***(Timothy et al., 2009).***

Epidemiology:

Asthma is a serious global health problem. People of all ages throughout the world are affected by this chronic airway disorder that when uncontrolled, can place severe limits on daily life and is sometimes fatal. The prevalence of asthma is increasing in most countries, especially among children. The burden of asthma is experienced not only in terms of healthcare costs but also as lost productivity and reduced participation in family life ***(Bateman et al., 2008).***

The prevalence of asthma has increased significantly since the 1970s. In 2009, 300 million people were affected worldwide. In 2009 asthma caused 250,000 deaths globally ***(Liard et al., 2009).***

Immunopathogenesis:

A variable degree of broncho obstruction based on AHR and allergen-dependent mast cell degranulation together with chronic airway eosinophilia, increased mucus production, and airway remodeling sketches the typical pathologic picture of allergic bronchial asthma ***(Bousquet et al., 2000).***

This complex phenotype is believed to arise from manifold interactions of infiltrating immune cells with structural cells of the airways. These processes comprise a plethora of cells such as T cells, B cells, mast cells, and

macrophages on the one hand and smooth muscle cells, fibroblasts, and airway epithelial cells on the other hand ***(Wegmann, 2009).***

Under physiological circumstances, the epithelium forms a highly regulated and almost impermeable barrier through the formation of tight junctions. The epithelial cell layer acts as a molecular sieve that excludes inhaled antigens and pathogens. However, some antigens can be recognized by cells of the immune system and induce an immune response. Mucosal dendritic cells (DCs) are extremely efficient sentinels in the defense against antigen challenge. They are strategically positioned within the epithelium in the basolateral space, separated from the inhaled air only by the epithelium tight junction barrier. However, DCs extend their processes between epithelial cells directly into the airway lumen. This "periscope" function provides a mechanism for continuous immune surveillance of the airway luminal surface ***(Jahnsen et al., 2006), Figure 1 (Hammad and Lambrecht, 2008).***