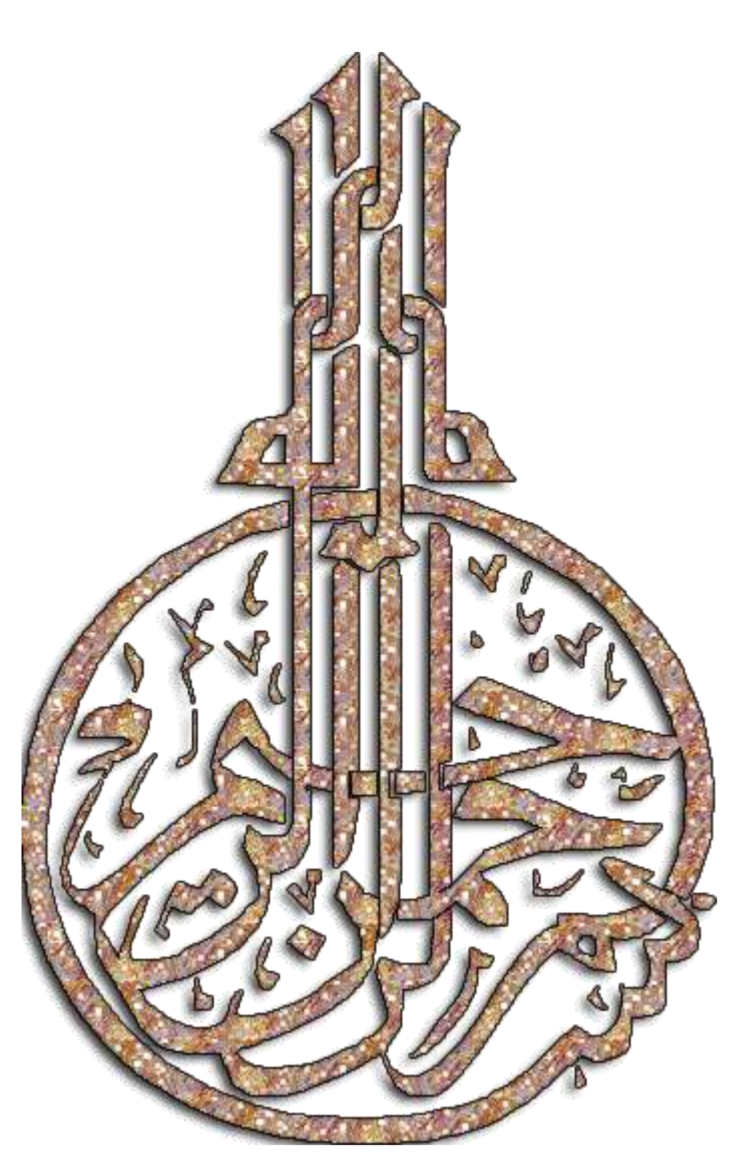
Psychological Stress and The Immune System in Female University Students

By
Afrah Abdullah Eskandar

A thesis submitted for the requirements of the degree of Master of Science (Biochemistry)

Supervised By
Dr. Sawsan H. Mahassni

Faculty of Science
King Abdulaziz University
Jeddah-Saudi Arabia
Jumada Awal 1434H – April 2013G



Psychological Stress and The Immune System in Female University Students

By
Afrah Abdullah Eskandar

A thesis submitted for the requirements of the degree of Master of Science (Biochemistry)

Supervised By
Dr. Sawsan H. Mahassni

FACULTY OF SCIENCE
KING ABDULAZIZ UNIVERSITY
JEDDAH-SAUDI ARABIA
JUMADA AWAL 1434H – APRIL 2013G

Psychological Stress and The Immune System in Female University Students

By
Afrah Abdullah Eskandar

A thesis submitted for the requirements of the degree of Master of Science (Biochemistry)

EXAMINATION COMMITTEE

	Name	Rank	Field	Signature
Internal Examiner	Dr. Karima S. Mohamed	Associate Professor	Biochemistry	[signature]
External Examiner	Dr. Sahar R. El- Hadad	Assistant Professor	Immunology and Molecular Biology	[signature]
Principal Supervisor	Dr. Sawsan H. Mahassni	Assistant Professor	Immunology	Sawsan Mahassni

KING ABDULAZIZ UNIVERSITY
Jumada Awal 1434H – April 2013G

Dedication

This Project is dedicated to my parents, my husband, my son, my brothers and sisters

(May God bless them all)

ACKNOWLEDGEMENT

First, I thank Allah for helping me to finish my studies and my research.

Special thanks to my major supervisor Dr. Sawsan H. Mahassni for her supervision and guidance.

I wish to thank Mrs. Hanadi Mansor for her help in the statistical section of my research.

I would like to thank all those who helped me in King Fahd Center for Medical Research with special thanks to Dr. Jummanah M. Jarullah.

My sincere thanks to my father and my mother who supported me especially in the hard times, to my husband who encouraged me and to my brothers and sisters.

Finally, I would like to thank to King Abdulaziz City for Science and Technology for its financial support to this research (أط-١٠-٠٠٧٤).

شكر و تقدير

أتقدم بخالص الشكر و التقدير لمدينة الملك عبد العزيز للعلوم و التقنية لدعمها المالي لمشروع البحث رقم (أط-١٠-٠٠٧٤) مما مكنني من توفير اللوازم والمواد الكيميائية الضرورية للبحث.

Extend my sincere thanks and appreciation to the King Abdul Aziz City for Science and Technology for their financial support for the research project No (P-S-10-0074) which enabled me to provide supplies and chemicals necessary to the research.

Psychological Stress and the Immune System in Female University Students

Afrah Abdullah Eskandar

Abstract

A wealth of research indicates that psychological stress suppresses various parameters of immune functions and consequently can cause diseases. Various studies suggested that acute stress enhances immunity whereas chronic stress suppresses immune functions. One type of acute psychological stress is examination stress which presents as a specific condition of anxiety and tension during examination period. This research studies the effects of examination stress on the immune system in forty one female university students. Blood samples were collected and the immune parameters and lipid profile were measured during the final examination period and during the non final examination period. The analysis of the results was divided into three sections. In the first section, the results of the two periods were compared. In the second, section the students of the two periods were divided into low, intermediate and high stress level groups according to their scores on the perceived stress questionnaire. In the third section, the students of the two periods were divided into low and high stress level groups according to the questionnaire.

In the first section, white blood cells, neutrophils, lymphocytes and cortisol hormone increased highly significantly in the examination period. The Red blood cells and IgM antibodies increased significantly, while the IgG antibodies decreased highly significantly. In the second section, the same trends found in the first section were also seen here in the examination period with additional significant increases in monocytes, and eosinophils and no change in the IgM concentration. In the third section, the differences observed compared to the first section were a highly significant increase in basophils and no change in the IgM concentration. In conclusion, this study, especially dependent on section one without using questionnaire, revealed that acute psychological stress during examination period enhanced the immunity of the students. It is recommended that further studies should be carried using a larger sample and using male university students or high school students.

الضغط النفسي و الجهاز المناعي لدى طالبات الجامعة

أفراح عبد الله إسكندر

المستخلص

هنـاك ثـروة مـن الأبحـاث التـي تشير إلـى أن الضـغط النفسـي يقمـع مختلـف معلمـات الوظـائف المناعيـة و بالتـالي يمكـن أن يسبب الأمـراض. العديـد مـن الدراسـات تشـير إلـى أن الضغط النفسي الحاد يعزز المناعة بينما الضغط النفسي المزمن يقمع الوظائف المناعية. احد أنواع الضغط النفسي الحاد هو ضغط الامتحانات والذي يمثل حالة خاصة من القلق و التوتر أثناء فترة الامتحانات. هذا البحث يدرس تأثير ضغط الامتحانات على الجهاز المناعي لإحدى وأربعين من طالبات الجامعة. تم جمع عينات الدم وقياس المعلمات المناعية والدهون خلال فترة الامتحانات النهائية و فترة عدم وجود امتحانات نهائية، و تمت مقارنة نتائج الفترتين في القسم الأول من تحليل النتائج. في القسم الثاني تم تقسيم الطالبات في كلتا الفترتين إلى ثلاث مجموعات: مجموعة مستوى الضغط النفسي المنخفض، مجموعة مستوى الضغط النفسي المتوسط ومجموعة مستوى الضغط النفسي العالي وفقا لاستبيان الضغط المحسوس وتم مقارنة نتائج كل مستوى للفترتين. في القسم الثالث تم تقسيم الطالبات في كلتا الفترتين إلى مجموعتين مجموعة مستوى الضغط النفسي المنخفض و مجموعة مستوى الضغط النفسي العالي وفقا لاستبيان الضغط المحسوس وتم مقارنة نتائج كل مستوى للفترتين.

في القسم الأول، خلال فترة الامتحانات لوحظ أن هناك زيادة معنوية كبيرة في خلايا الدم البيضاء، الخلايا المتعادلة الاصطباغ، الخلايا اللمفاوية و هرمون الكورتيزول. ولوحظ أن هناك زيادة معنوية في خلايا الدم الحمراء و تركيز الأجسام المضادة M بينما كان هناك انخفاض معنوي كبير في تركيز الأجسام المضادة G. في القسم الثاني، شوهدت نتائج مماثلة للقسم الأول في فترة الامتحانات بالإضافة إلى زيادة معنوية في الخلايا أحادية النوى والخلايا القاعدية الاصطباغ ولم يوجد أي تغير في تركيز الأجسام المضادة M. في القسم الثالث، كانت الاختلافات التي لوحظت مقارنة بالقسم الأول زيادة معنوية كبيرة في الخلايا قاعدية الاصطباغ ولم يوجد تغير في تركيز الأجسام المضادة M. يستنتج من هذا أن هذه الدراسة، خصوصا بالاعتماد على القسم الأول بدون استخدام الاستبيان، كشفت أن الضغط النفسي الحاد أثناء فترة الامتحانات عزز المناعة لدى الطالبات. لمزيد من الدراسات في هذا الموضوع نوصي بأن تكون العينة أكبر و يمكن تطبيق الدراسة على عينات أخرى مثل طلاب الجامعة الذكور أو طلاب المدارس الثانوية.

TABLE OF CONTENTS

Examination Committee Approval…………………………………………...
Dedication……………………………………………………………….....
Acknowledgement ………………………………………………………. iv
Abstract………………………………………………………………... vi
Arabic Abstract ………………………………………………………. vii
Table of Contents ……………………………………………………….. viii
List of Figures ………………………………………………………… xii
List of Tables ………………………………………………………. xiii
List of Symbols and Terminology……………………………………… xvi

Chapter I: Introduction …………………………………………………... 1
1.1 Stress………………………………………………………………….. 1
1.1.1 Types of stress…………………………………………………….. 2
1.1.2 Stressors ……………………………………………………………... 3
1.1.3 Differences between ethnic groups or countries …………………………. 3
1.1.4 The effects of stress on health…………………………………………….. 5
1.1.4.1 Stress and the nervous system……………………………………… 6
1.1.4.2 Stress and the cardiovascular system ……………………………… 6
1.1.4.3 Stress and obesity………………………………………………… 7
1.1.4.4 Stress and diabetes ……………………………………………… 8
1.1.4.5 Stress and immune system ……………………………………… 8
1.1.5 Measurement of stress …………………………………………………. 9
1.1.5.1 Physiological measurements…………………………………….... 9
1.1.5.1.1 Cortisol………………………………………………… 10
1.1.5.2 Psychological measurements……………………………………... 11
1.1.5.2.1 The Perceived Stress Questionnaire……………………… 11

1.2 The immune system ………………………………………………………….. 12
1.2.1 Types of immunity …………………………………………………...... 13
1.2.1.1 Innate immunity ………………………………………………………. 13
1.2.1.2 Adaptive immunity …………………………………………………… 14
1.2.2 Organs of the immune system ………………………………………… 14
1.2.3 Cells and molecules of the immune system ……………………………... 15
1.2.3.1 Cells of the lymphoid series ……………………………………… 15
1.2.3.1.1 T lymphocytes……………………………………………... 15
1.2.3.1.2 B lymphocytes …………………………………………….. 15
1.2.3.1.2.1 Immunoglobulin M ………………………………. 18
1.2.3.1.2.2 Immunoglobulin G ………………………………. 18
1.2.3.1.2.3 Immunoglobulin E ……………………………….. 19
1.2.3.1.2.4 Immunoglobulin A ………………………………. 19
1.2.3.1.2.5 Immunoglobulin D ………………………………. 20
1.2.3.2 Cells of the myeloid series ……………………………………….. 21
1.2.3.2.1 Leukocytes ………………………………………………... 21
1.2.3.2.1.1 Neutrophils ………………………………………... 22
1.2.3.2.1.2 Eosinophils ……………………………………...... 22
1.2.3.2.1.3 Basophils…………………………………………... 23
1.2.3.2.1.4 Monocytes ………………………………………… 23
1.2.3.2.2 Thrombocytes (Platelets)………………………………….. 24
1.2.3.2.3 Erythrocytes (Red blood cells) ……………………………… 25
1.2.3.3 Cytokines …………………………………………………………. 25
1.2.3.3.1 Interleukin-6 ………………………………………………… 26
1.3 Lipid profile…………………………………………………………....... 27
1.3.1 Cholesterol ……………………………………………………………... 27
1.3.2 Triglycerides …………………………………………………………… 28
1.3.3 Low density lipoprotein ………………………………………………….. 28
1.3.4 High density lipoprotein …………………………………………………. 29
1.4 The relationship between stress and the immune system …………………… 30
1.4.1 The relationship between stress and blood cells……………………….... 32
1.4.2 The relationship between stress and Antibodies………………………….. 36
1.4.3 The relationship between stress and Interleukin-6……………………….. 39

1.5 The relationship between stress and cortisol…………………………………….. 41
1.6 The relationship between stress and the lipid profile…………………………. 43
1.7 Aim of the study…………………………………………………………………… 47

Chapter II: Materials and Methods…………………………………………….. 49
2.1 Subjects and Anthropometric Measurements……………………………….... 49
2.1.1 Categorizations of Subjects ……………………………………………… 50
2.1.2 Blood Collection…………………………………………………………….. 51
2.1.3 Parametric Analysis………………………………………………………….. 52
2.1.3.1 Differential Complete Blood Count………………………………… 52
2.1.3.2 Determination of Serum Triglycerides Concentration……………… 52
2.1.3.3 Determination of Total Serum Cholesterol Concentration………….. 54
2.1.3.4 Determination of Serum HDL-Cholesterol Concentration…………. 55
2.1.3.5 Determination of Serum LDL-Cholesterol………………………….. 56
2.1.3.6 Quantitative Determination of Total Serum Cortisol Concentration . 57
2.1.3.7 Qualitative Determination of Serum IgG and IgM Concentrations… 58
2.1.3.8 Quantitative Determination of Serum IgA, IgE and IgD Concentrations……………………………………………………… 60
2.1.3.9 Quantitative Determination of Serum Interleukin-6 Concentration… 61
2.2 Statistical methods ………………………………………………………………… 62
2.2.1 Descriptive statistics …………………………………………………………… 62
2.2.2 Analytical statistics……………………………………………………………... 63

Chapter III: Results………………………………………………………………... 64
3.1 Section one: Uncategorized comparisons of the two cases…………………… 64
3.1.1 The differential complete blood count test……………………………….... 64
3.1.2 Serum levels of the lipid profile ………………………………………….. 66
3.1.3 Serum levels of cortisol and IL-6 ……………………………………….... 66
3.1.4 Serum levels of IgE, IgA, IgD, IgM and IgG antibodies…………………. 66
3.2 Section two: Categorized comparisons of the parameters (three categories)…. 71
3.2.1 The differential complete blood count test ……………………………….. 72
3.2.2 Serum lipid profile ……………………………………………………….... 77
3.2.3 The serum levels of cortisol and IL-6 ……………………………………. 77

3.2.4 Serum levels of IgE, IgA, IgD, IgM and IgG antibodies ………………… 81
3.2.5 Comparison of the average of the 3 groups of the two cases at normal and non-normal distributions for the parameters…………………………. 84
3.2.5.1 Comparison of the average of the 3 groups of the two cases at normal and non-normal distributions for CBC………………....... 84
3.2.5.2 Comparison of the average of the 3 groups of the two cases at normal and non-normal distributions for lipid profile……………. 84
3.2.5.3 Comparison of the average of the 3 groups of the two cases at normal and non-normal distributions for cortisol and IL-6……… 84
3.2.5.4 Comparison of the average of the 3 groups of the two cases at normal and non-normal distributions for IgE, IgA and IgD……... 88
3.3 Section three: Categorized comparisons of the parameters (two categories)…. 90
3.3.1 The differential complete blood count……………………………………. 91
3.3.2 Serum lipid profile………………………………………………………… 95
3.3.3 The serum levels of cortisol and IL-6 ……………………………………. 97
3.3.4 Serum levels of IgE, IgA, IgD, IgM and IgG antibodies ………………… 97
3.3.5 Comparison of the average of the two stress groups of the two cases at normal and non-normal distributions for the parameters……………… 101
3.3.5.1 Comparison of the average of the two stress groups of the two cases at normal and non-normal distributions for CBC…………….. 101
3.3.5.2 Comparison of the average of the two stress groups of the two cases at normal and non-normal distributions for lipid profile…….. 101
3.3.5.3 Comparison of the average of the two stress groups of the two cases at normal and non-normal distributions for cortisol and IL-6. 104
3.3.5.4 Comparison of the average of the two stress groups of the two cases at normal and non-normal distributions for IgE, IgA and IgD 104

Chapter VI: Discussion ………………………………………………………. 107
Chapter V: Conclusion and Recommendations……………………………….. 117
List of References ……………………………………………………………… 118
Appendices …………………………………………………………………… 133
Arabic Summary

LIST OF FIGURES

Figure **Page**

1.1 Cells of the immune system …………………………………… 16

LIST OF TABLES

Table **Page**

Table		Page
3.1	The differential CBC for uncategorized comparisons of the two cases using paired samples t-test…………………………………	65
3.2	Serum levels of the lipid profile for the uncategorized comparisons of the two cases using paired samples t-test………..	67
3.3	Serum levels of cortisol and IL-6 for the uncategorized comparisons of the two cases using paired samples t-test………..	68
3.4	Serum levels of IgE, IgA and IgD for the uncategorized comparisons of the two cases using paired samples t-test…….….	69
3.5	Serum levels of IgM and IgG for the uncategorized comparisons of the two cases using Fisher's test………………………………	70
3.6.a	The differential complete blood count test results for the categorized (three groups) comparisons of the two cases using paired samples t-test……………………………………………	73
3.6.b	The differential complete blood count test results for the categorized (three groups) comparisons of the two cases using paired samples t-test……………………………………………	74
3.6.c	The differential complete blood count test results for the categorized (three groups) comparisons of the two cases using paired samples t-test……………………………………………	75
3.7.a	The serum levels of lipid profile results for the categorized (three groups) comparisons of the two cases using paired samples t-test……………………………………………........................	78
3.7.b	The serum levels of the lipid profile for the categorized (three groups) comparisons of the two cases using paired samples t-test..………………………………………………………	79

Table		Page

3.8	The serum levels of cortisol and IL-6 results of for the categorized (three groups) comparisons of the two cases using paired samples t-test……………………………………………	80
3.9	Serum levels of IgE, IgA and IgD for the categorized (three groups) comparisons of the two cases using paired samples t-test……………………………………………………………...	82
3.10	Serum levels of IgM and IgG for the categorized comparisons (three groups) of the two cases using Fisher's test……………….	83
3.11	Comparison of the average of the 3 groups of the two cases at normal and non-normal distributions for the differential complete blood count test……………………………………...................	85
3.12	Comparison of the average of the 3 groups of the two cases at normal and non-normal distributions for lipid profile……………	86
3.13	Comparison of the average of the 3 groups of the two cases at normal and non-normal distributions for cortisol and IL-6………	87
3.14	Comparison of the average of the 3 groups of the two cases at normal and non-normal distributions for IgE, IgA and IgD……..	89
3.15.a	The differential complete blood count test results for the categorized (two groups) comparisons of the two cases using paired samples t-test…………………………………………...	92
3.15.b	The differential complete blood count test results for the categorized (two groups) comparisons of the two cases using paired samples t-test……………………………………………	93
3.15.c	The differential complete blood count test results for categorized (two groups) comparisons of the two cases using paired samples test……………………………………………………………...	94
3.16	The serum levels of the lipid profile for the categorized (two groups) comparisons of the two cases using paired samples t-test……………………………………………………………...	96
3.17	The serum levels of cortisol and IL-6 for the categorized (two groups) comparisons of the two cases using paired samples t-test……………………………………………………………...	98

Table **Page**

3.18 Serum levels of IgE, IgA and IgD for the categorized (two groups) comparisons of the two cases using paired samples t-test…………………………………………………………...... 99

3.19 Serum levels of IgE, IgA and IgD for the categorized comparisons (two groups) of the two cases using Fisher's test….. 100

3.20 Comparison of the average of the two stress groups of the two cases at normal and non-normal distributions for CBC………… 102

3.21 Comparison of the average of the two stress groups of the two cases at normal and non-normal distributions for lipid profile….. 103

3.22 Comparison of the average of the two stress groups of the two cases at normal and non-normal distributions for cortisol and IL-6……………………………………………………………….... 105

3.23 Comparison of the average of the two stress groups of two cases at normal and non-normal distributions for IgE, IgA and IgD….. 106

LIST OF SYMBOLS AND TERMINOLOGY

4-AA	4-Aminoantipyrine
ACTH	Adrenocorticotropin hormone
Apo B	Apolipoprotein B
ATP	Adenosine-5-triphosphate
BMI	Body mass index
CBC	Complete blood counts
CBSM	Cognitive-behavioral stress management
CE	Cholesterol esterase
CIC	Circulating immune complexes
CNS	Central nervous system
CO	Cholesterol oxidase
CRF	Corticotropin-releasing factor
CVD	Cardiovascular disease
DEA-HCL/AAP	N,N-diethylaniline-HCL/4-aminoantipyrine
DSBmT	N,N-bis(4-sulphobutyl)-m-toluidine-disodium salt
EDTA	Eethylene diamine tetra-acetic acid
ELISA	Enzyme-linked immunosorbent assay
g/dL	Grams per deciliter
GK	Glycerol kinase
GPO	Glycerol-3-phosphate–oxidaze

H_2O_2	Hydrogen peroxide
HDL	High density lipoprotein
HPA	Hypothalamic–pituitary–adrenal
HPO	Horseradish peroxidase
Hu IL-6	Human Interleukin-6
Ig	Immunoglobulins
Ig A	Immunoglobulin A
IgD	Immunoglobulin D
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL2R	Interleukin-2 receptor
IL-6	Interleukin-6
IU/mL	International units per milliliter
LDL	Low density lipoproteins
LPL	Lipoprotein lipase
mg/dL	Milligram per deciliter
mmol/L	Millimoles per litre
ng/mL	Nanogram per milliliter
OD	(Optical density) Absorbance
pg/mL	Picograms per milliliter
PNI	Psychoneuroimmunology
POD	Peroxidase
PSQ	Perceived Stress Questionnaire
PSS	Perceived stress scale

PTSD	Posttraumatic stress disorder
RBC	Red blood cell
SD	Standard deviation
SE	Standard error
S-IgA	Secretory immunoglobulin A
TAG	Triacylglycerols
TG	Triglycerides
TMB	Tetramethylbenzidine
WBC	White blood cell
WC	Waist circumference
WHO	The World Health Organizations
WHR	Waist-to-hip ratio
μg/dL	Microgram per deciliter
μl	Microliter

CHAPTER I

Introduction

Chapter I

Introduction

1.1 Stress

The word 'stress' was first used in its current meaning and became popular by the Hungarian Canadian experimentalist Hans Selye in 1956 (Chrousos, 2009). Stress may be defined as a threat to the organism's homeostasis, reflecting the need to maintain stability through change, and it may be a physical assault or emotional burden which activates neuronal, hormonal and behavioral programs to maintain or restore the homeostasis (Van Houdenhove and Egle, 2004). Another definition of stress is a feeling that is created when one reacts to particular events. Stress is the body's way of rising to a challenge and preparing to meet a tough situation with focus, strength, stamina, and heightened alertness. Stress results when something causes the body to behave as if it were under attack (Mitra, 2008). Psychological stress is usually defined as the experience of negative events or the perceptions of distress and negative affects that are associated with the inability to deal with them (Cohen *et al*, 2001).

Prolonged stress can lead to depression because prolonged elevated cortisol that results from chronic stress can be associated with serotonin (important neurotransmitter in mood regulation) dysfunctions that results in depression

(Sharpley, 2009). Life stress, biochemical factors, such as illness infection, certain drugs and genetic factors, including a family history of depression, may play a role in some cases of depression (Brigitta, 2002).

Another factor leading to depression and increased stress is sleep deprivation. As a result of a more demanding life style, the average time available for nightly sleep is decreasing in humans (Sgoifo *et al*., 2006). Sleep deprivation results due to the impact of life stressors, such as increased work load, shift work, and various other stressors that are induced by modern society and may represent a serious threat for health. There are different potential harmful effects of sleep disturbances, one of which is cardiovascular diseases (Sgoifo *et al*., 2006).

1.1.1 Types of stress

There are two types of stress according to its duration and intensity. The first type of stress is acute or short-term stress, which is defined as stress that lasts for a period of minutes to hours in duration. The second type of stress is chronic or long-term stress, which is defined as stress that persists for several hours per day for weeks or months (Dhabhar, 2008). The intensity of stress may be known by levels of stress hormones, neurotransmitters, and other physiologic changes, such as increases in heart rate and blood pressure, and by the amount of time for which these changes continue during stress and following the cessation of stress (Dhabhar, 2008).

Chronic stress suppresses or dysregulates innate and adaptive immune responses through the suppression of antibody production, leukocyte numbers, trafficking, and function or changes in the cytokine balance. Chronic stress also increases susceptibility to skin cancer (Dhabhar, 2008). In contrast to chronic

stress, which is generally harmful, acute stress activates the immune system and antigen exposure induces a redistribution of circulating immune cells to the organs of the body (Dhabhar *et al*., 2010). Acute stress may help to prepare the immune system for potential challenges, such as wounding or an infection, which may serve as an early warning signal by the brain and also it induces enhancement of skin immunity (Dhabhar, 2008).

1.1.2 Stressors

The existence of stress depends on the existence of stressors. Stressors are environmental, physical, mental, or emotional factors that challenge an individual's adaptability or stimulate an individual's body or mentality (Kai-Wen, 2009). There are two types of stressors, physical stressors (such as exercises, alcohol abuse, drug abuse, smoking, pain and illness) and psychological stressors (such as exams, divorce, moving and the death of a loved one) (Whitney and Rolfes, 2005). Both physical stressors (also known as external stressors) and psychological stressors (also known as the internal stressors) pose a challenge to homeostasis (Dhabhar, 2008). Physical stressors lead to the onset of diseases directly. Psychological stressors are thought to influence the pathogenesis of physical diseases by causing negative affective states (such as feeling of depression and anxiety), which in turn exert direct effects on biological processes or behavioral patterns (such as smoking, decreased exercises and sleep) that influence the disease risk (Cohen *et al*., 2007).

1.1.3 Differences between ethnic groups or countries

Job related stress has been steadily increasing for decades in the United States and other industrial countries**.** The World Health Organizations (WHO) has

confirmed that work related stress has become a “world-wide epidemic” reaching far beyond the United States and other industrial countries (Bahrami, 2010). In 1992, The United Nations described work stress as “The Twentieth-Century Disease”. In 2006, Chinese businessmen experienced an increase in stress levels by 84%, followed by the Taiwanese who exhibited an 82% increase, Indians 79%, and Russians 76%. A European survey conducted in the late nineties found that more than half of the 147 million workers in the European Union complained from the aggressive deadlines of work, which cause them to experience higher levels of stress (Bahrami, 2010). An extensive survey among a sample of 15,800 workers from 15 countries of the European Union, showed that 28% of the workers suffered from work-related stress, and 20% reported overall fatigue as a work-related health problem (Kompier *et al*., 2000).

A study on work stress and racial discrimination among minority ethnic workers in the United Kingdom showed that a higher percentage of black African–Caribbean respondents reported high work stress compared to Bangladeshi and white respondents, and the racial discrimination among black African–Caribbean respondents was strongly associated with high perceived work stress (Wadsworth *et al*., 2007).

Stress and coping models are potentially useful for multicultural research because they are interested in the effects of social environmental factors on human functioning. Multicultural models of the stress process include a number of cultural factors that influence the stress model (Slavin *et al*., 1991). A multicultural model of the stress process was used as the basis for exploring ethnic and racial differences in the life stress process among 103 black, 129 Hispanic, and 105 white students from a multiethnic predominantly minority high school. White

adolescents reported more negatively impactful stressful life events than did Black or Hispanic adolescents (Slavin *et al.*, 1991). Minority status predicted ethnic (sociological factors such as culture, language and beliefs) and racial (biological factors such as skin color, eye color) differences independent of socioeconomic status (Prelow and Guarnaccia, 1997). Results also indicated that racial identity attitudes were related to the stress associated with experiences of racism at the cultural and individual level, whereas ethnic identity was not associated to race-related stress. Ethnic identity did not moderate the relation of racial identity to race-related stress (Samon and Consuelo, 2006).

1.1.4 The effects of stress on health

Under unstressful conditions, the hypothalamic–pituitary–adrenal (HPA) axis acts normally and results in normal levels of cortisol, which are important for normal brain growth and support metabolic activity necessary to maintain general functioning of the body (Bevans *et al.*, 2008). Under stressful conditions, physical or psychological stress activates the HPA axis and leads to elevation of cortisol levels. Although short-term elevations in cortisol levels can be critical for survival in stressful circumstances, long exposure to elevated cortisol levels can lead to health risks including muscle atrophy, decreased sensitivity to insulin, hypertension, cardiovascular disease, impaired growth and tissue repair and neurological damage. Also, chronic exposure to low levels of cortisol can cause health risks including asthma, allergies, disorders related to hyperactivity of the immune system, and neuronal damage (Bevans *et al.*, 2008).

1.1.4.1 Stress and the nervous system

Stress has effects on the learning and memory areas of the brain which result in long-term problems with learning and memory that have a neurological basis (Bremner, 2004). The brains of stress patients resemble patients with organic memory problems (which are caused by physical trauma or diseases) or early dementia (which is caused by old age). Posttraumatic stress disorder (PTSD) described as a disorder in which there is accelerated aging, which may include both an acceleration of the memory deficits that are seen in some of the elderly as well as an increased risk for stroke, heart disease and diabetes (Bremner, 2004). Stress also leads to stimulation of arousal and suppression of sleep (Chrousos, 2009). Elevated levels of cortisol lead to depression and feelings of fatigue by affecting mood (Bremner, 2004).

1.1.4.2 Stress and the cardiovascular system

Stress may cause heart disease directly, by activation of neuroendocrine responses to stressors that activate the autonomic nervous system which lowers heart rate variability or by leading to the metabolic syndrome and incidence of obesity which cause heart diseases. Stress may cause heart disease indirectly, through unhealthy behaviors that increase the risk of heart disease, such as smoking, lack of exercise, or excessive alcohol consumption (Chandola *et al.*, 2008). The stress hormone cortisol adversely affects atherosclerosis leading to sudden death. Depression is related to stress in many cases, and it has been shown that patients with depression and heart disease are about five times more likely to have sudden death relative to patients with heart disease without depression (Bremner, 2004). Mental stress is reported to induce myocardial ischemia in

patients with coronary artery disease via alterations in both myocardial oxygen demand and supply (Merz *et al*., 1993). Chronic fatigue, which is related to stress, can lead to sudden cardiac death in both healthy people and patients with heart disease. One form of chronic fatigue is chronic sleep deprivation (Takase *et al*., 2004).

1.1.4.3 Stress and obesity

Elevations in cortisol levels have been shown to result in increased deposition of intraabdominal fat, which is associated with a higher mortality than obesity in general (Bremner, 2004). Psychological stress causes metabolic changes, such as an elevated corisol level that causes an increase in the level of insulin hormone leading to an increase of blood glucose, which subsequently leads to energy storage in abdominal fat depots. Abdominal obesity may promote cellular aging and earlier cell senescence (Epel, 2009).

Central components of the stress system are closely linked to brain centers that control appetite and energy expenditure. Obesity progressively leads to metabolic complications, such as diabetes, dyslipidemia, hypertension and cardiovascular disease. Increased intake of processed food, a sedentary lifestyle and increased psychological stress are characteristics of the modern way of life in industrialized societies and they are forming an environment that differs completely from that of our predecessors. Thus, it is not surprising that the rates of obesity and stress-related complications have been increasing over the past decades (Kyrou and Tsigos, 2008).

1.1.4.4 Stress and diabetes

There is no evidence that stress causes diabetes. However, stress may sometimes unmask diabetes, by elevating blood glucose levels. In a research study, mental stress and physical stress, such as illness or injury, resulted in increasing blood glucose levels in people of the two types of diabetes. It was found that controlling stress with relaxation therapy can help these patients (Mitra, 2008). Stress prevents the release of insulin in people with Type 2 diabetes and reducing stress may be more helpful for these people. Stress reduction in people with Type 1 diabetes does not have this effect because they do not make insulin, but reducing stress can help these people to take better care of their health (Mitra, 2008).

1.1.4.5 Stress and immune system

Stress suppresses the immune system and its functions, which can lead to an increase in the susceptibility to infections and cancer (Bremner, 2004). Stress has complex effects on the immune system and affects innate and acquired immunities. Glucocorticoids (a group of steroid hormones that are involved in carbohydrate, protein, and fat metabolism and have anti-inflammatory properties; such as cortisol) and catecholamines (a group of amines derived from catechol that have important physiological effects as neurotransmitters and hormones and include epinephrine, norepinephrine, and dopamine) influence trafficking and function of leukocytes, mononuclear phagocytes and dendritic cells and suppress the secretion of proinflammatory cytokines (Chrousos, 2009).

Psychological stress is also known to increase some allergic, autoimmune, and inflammatory diseases, which suggests that stress may enhance immune functions under certain conditions (Dhabhar, 2008). A stress response involves the

release of neurotransmitters and hormones in the body and the response is generally adaptive in the acute stress and damaged in chronic stress. The body responds to warning signals that are sent by the brain, preparing the body to deal with the consequences of stress in the case of acute stress which can affect immune cells trafficking, maturation, or function in ways that can enhance immunity and prepare the immune system for challenges (such as wounding or infection) that may be induced by a stressor (Dhabhar *et al*., 2010). While acute stress enhances immune function, chronic stress often suppresses or dysregulates immune function (Dhabhar, 2008). When the acute stress continues, it will be converted to chronic stress because of the continuous increases in stress hormones that resulte in suppression of the immune cells and an increased risk of infections (Aronson, 2009).

1.1.5 Measurement of stress

There are two approved ways for the measurement of stress, which are physiological measurements and psychological measurements (Haubenhofer and Kirchengast, 2007), as described below.

1.1.5.1 Physiological measurements

During stressful conditions the hypothalamic-pituitary-adrenal (HPA) gland plays an important function in the response. The hypothalamus releases corticotropin-releasing factor (CRF) during stress, which increases the secretion of adrenocorticotropin hormone (ACTH) from the anterior pituitary, which in turn stimulates the production of glucocorticoids which include stress hormones (cortisol and corticosterone) from the adrenal cortex (Bremner *et al*., 2003).

Cortisol and corticosterone and their metabolites can be measured in blood, tissues, urine, saliva and feces. Cortisol is mostly used as an indicator and biomarker of stress in many stress studies (Bayazit, 2009) and its measurement can help identify stressors and the efficacy of interventions aimed at reducing stress (King and Hegadoren, 2002).

1.1.5.1.1 Cortisol

The main glucocorticoid hormone in humans is cortisol, which is a catabolic hormone secreted from the adrenal cortex in response to psychological and physical stress (Brownlee *et al*., 2005). Human cortisol is commonly known as a 'stress hormone' due to the increase of cortisol release in response to physiological or psychological stress (Putman and Roelofs, 2011). Also life events and difficulties can cause cortisol hypersecretion but not necessarily lead to the development of depression (Cowen, 2002). The concentration of cortisol in the blood averages 12 μg/100 ml and about 90% to 95% of the cortisol in the plasma binds to plasma proteins called cortisol-binding globulin or transcortin and, to a lesser extent, to albumin. This high degree of binding slows the removal of cortisol from the plasma and gives it a long half life of 60 to 90 minutes (Guyton and Hall, 2006).

Cortisol has many functions including controlling the metabolism of proteins, carbohydrates, and fats, and it also has an anti-inflammatory function. After the incidence of inflammation, the administration of cortisol can reduce inflammation within hours to a few days and immediately block most of the factors that increase inflammation (Guyton and Hall, 2006). Also the rate of healing is increased in the case of cortisol administration, which may be due to the

mobilization of amino acids that repair the damaged tissues, the enhanced glucogenesis that increases glucose available in critical metabolic systems, the increased amounts of fatty acids available for cellular energy; or due to the effect of cortisol in inactivating or removing inflammatory products Cortisol has an anti-inflammatory effect because it blocks the release of histamine (which is produced by immune cells and acts as a neurotransmitter that activates inflammatory response) and prevents rupture of lysosomal membranes which leads to the protection of tissues (Guyton and Hall, 2006).

1.1.5.2 Psychological measurements

Questionnaires are important instruments used to get information about health, level of body functions in different situations, and level of experienced stress. Questionnaires are suitable tools for studying the outcomes of a large population and identifying the differences in these outcomes (O'Connor *et al.*, 2004).

The number of questionnaires that are interested in health status has increased over the past decades dramatically. As a result, it is more difficult to choose the questionnaire that is suitable for use in a particular situation, but recently a large number of reviews of available questionnaires, which are used to measure a specific concept in a specific population, have been published (Terwee *et al.*, 2007).

1.1.5.2.1 The Perceived Stress Questionnaire

Levenstein et al. in 1993 developed the "Perceived Stress Questionnaire" (PSQ) to assess stress situations on a mainly cognitive and emotional level. The PSQ aims to overcome some of the difficulties concerning the definition and

measurement of stress by putting the focus on the individual's subjective perception and emotional response (Fliege *et al.*, 2005). With this aim, item wordings were designed to represent the subjective perspective of the individual ("You feel. . . ") and the presented stress experiences were intended to be abstract enough to be applicable to adults of any age, stage of life, sex, or occupation. The PSQ was properly suitable and qualified for research on stress and illness (Fliege *et al.*, 2005).

The PSQ was originally published in English and then it was translated into German and other languages such as Spanish, Swedish and Dutch (Arck *et al.*, 2001). The German version of the PSQ has been validated and applied in healthy adults and different clinical populations (Rosenberger *et al.*, 2009). This questionnaire was developed for use in clinical psychosomatic research. In contrast to the original version of the PSQ with 30 items and 7 factors (harassment, overload, irritability, lack of joy, fatigue, worries, tension), an abbreviated German version comprises 20 items and 4 factors (worries, tension, joy, demands). The items can be answered with a four point rating scale (1 = almost never, 2 = sometimes, 3 = often, 4 = usually) (Kocalevent *et al.*, 2007).

1.2 The immune system

The immune system and its functions is one of the greatest wonders of the human body. It protects from environmental attacks faced from all sources, such as air, food or the other environmental agents that one is exposed to (Naik, 2003). The immune system is a network of tissues, cells and molecules, which act together to protect the body from invading infectious organisms (Staines *et al.*, 1994). The ability of the human body to resist different types of organisms or

toxins that can cause damage of its tissues and organs is called immunity (Guyton and Hall, 2006).

1.2.1 Types of immunity

There are two types of immunity, the innate (natural or non-adaptive) immunity and the adaptive (acquired or non-natural) immunity and they mainly differ in the speed and specificity of their reactions. The innate immunity is rapid, has no memory and lacks specificity. The adaptive immunity is specific, but takes several days or weeks to develop and has a memory, so that subsequent exposure leads to a stronger and more rapid response (Parkin and Cohen, 2001).

1.2.1.1 Innate immunity

Innate immunity provides the first and initial protection from a wide variety of foreign organisms, and it is naturally present (Mendes, *et al*., 2005). It includes physical, chemical, and cellular barriers (Parkin and Cohen, 2001). The physical barriers, such as the skin and mucous membranes lining the respiratory, gastrointestinal and genitourinary tracts, are the first defensive innate mechanisms which separate the organism from the environment (Blanco and Garcia, 2008). Chemical barriers include acids that are secreted by the stomach lining and enzymes found in mucus, tears, sweat, saliva and urine (Staines *et al*., 1994). Other components of innate immunity are the phagocytic cells (neutrophils, monocytes and macrophages), complement proteins, cytokines, and acute phase proteins, which provide rapid host defence (Parkin and Cohen, 2001).

1.2.1.2 Adaptive immunity

Adaptive immunity is induced specifically during infection and disease by adapting to antigens that cause the disease (Blanco and Garcia, 2008). Antigens are the substances that induce specific immune reactions and most of them are not normal constituents of the body (Staines *et al*., 1994).

There are two types of acquired immunity in the body, humoral immunity and cellular immunity. Humoral immunity is produced by B cells, which produce circulating antibodies in the blood plasma that are capable of attacking the foreign antigens. Cellular immunity, also termed cell-mediated or T-cell immunity, is achieved through the formation of large numbers of activated T lymphocytes that are specifically crafted in the lymph nodes and spleen to destroy antigens (Guyton and Hall, 2006).

1.2.2 Organs of the immune system

Organs of the immune system are divided into two types, primary lymphoid organs and secondary lymphoid organs, with the lymphatic vessels connecting the immune organs to the tissues and bloodstream. Primary immune organs are the sites where lymphocytes are produced, and include fetal liver, thymus and bone marrow (Guyton and Hall, 2006). Secondary lymphoid organs which include the spleen and lymph nodes are the sites where B and T cells are crafted and connected with antigen presenting cells in order to initiate an adaptive immune response (Ohl *et al*., 2003).

1.2.3 Cells and molecules of the immune system

Stem cells of the bone marrow are repeatedly and continuously dividing to give many daughter cells (Figure 1.1) that are undergoing differentiation processes to produce lymphoid and myeloid cells that are the common sources of various types of cells in the blood (Staines *et al.*, 1994).

1.2.3.1 Cells of the lymphoid series

1.2.3.1.1 T lymphocytes

After the formation of T lymphocytes in the bone marrow they migrate to the thymus gland where they divide rapidly and develop extreme diversity to react with different specific antigens. Then they leave the thymus and spread through the blood stream to the tissue of the site of infection (Guyton and Hall, 2006).

T cells can identify infected cells and play effector functions including direct cytotoxic effects on target cells or inhibiting the growth or survival of the pathogen by releasing cytokines (Kalia *et al.*, 2006). T cells are considered an important source of cytokines which play a vital role in regulating adaptive immune responses (Kristensen *et al.*, 2004).

1.2.3.1.2 B lymphocytes

B lymphocytes are cells of the immune system that express cell surface receptors called antibodies or immunoglobulins (Ig) which produce an immune response against specific antigens (LeBien and Tedder, 2008). B lymphocytes are formed in the liver during mid-fetal life and in the bone marrow during late fetal life and after birth (Guyton and Hall, 2006).

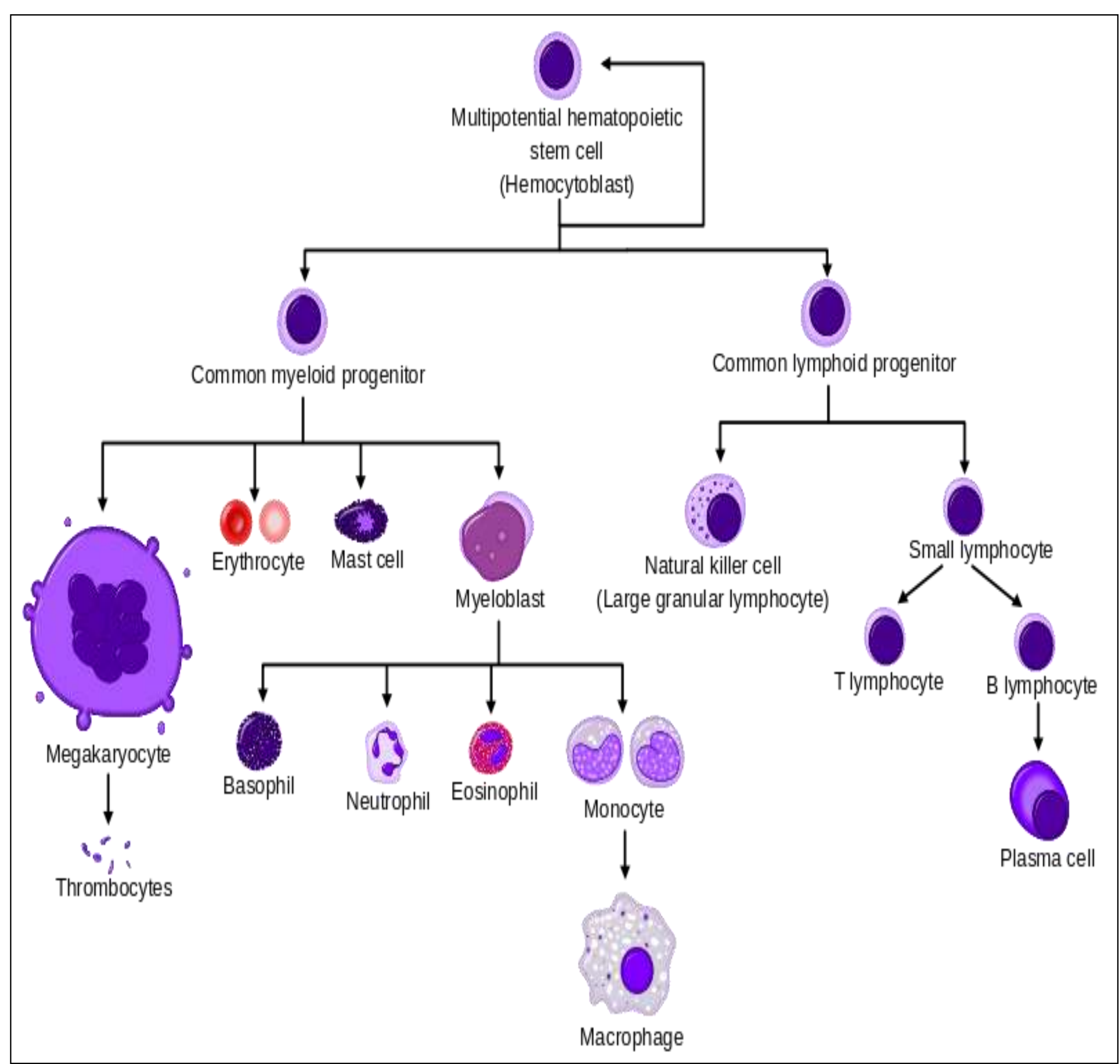


Figure 1.1 Cells of the immune system.

(http://en.wikipedia.org/wiki/Haematopoiesis)

Most antigens stimulate both T and B lymphocytes. T helper cells secrete specific substances, called lymphokines, which activate the specific B lymphocytes, which immediately enlarge and form lymphoblasts. Some of these lymphoblasts differentiate to form plasmablasts, which are precursors of plasma cells, and a few of the lymphoblasts form new B lymphocytes similar to those original B cells. After the formation of new B lymphocytes, they circulate throughout the body to the lymphoid tissue and they remain dormant until activated once again by the same antigen. Thus, they are called memory cells and they cause rapid and potent secondary immune response (Guyton and Hall, 2006).

On the surface of B lymphocytes there are antibodies, which trigger adaptive immune responses and control a series of antigen-independent checkpoints during B cell development (Meffre, *et al.*, 2000). Antibodies are considered a first line of defense against antigens and most vaccines work because they stimulate a protective antibody response (Gray *et al.*, 2007).

Antibodies are glycoproteins secreted by specialized B lymphocytes known as plasma cells. They are composed of four polypeptide chains with two identical copies of both a heavy and a light chain that are connected together by disulfide and non-covalent bonds (Lipman *et al.*, 2005). The antibody can be separated functionally into variable domains that bind antigens and constant domains that specify the functions and class of the antibody. There are five main classes of heavy chain constant domains and each defines one of the 5 classes of antibodies, which are the IgM, IgG, IgE, IgA, and IgD isotypes (Schroeder and Cavacini, 2010).

1.2.3.1.2.1 Immunoglobulin M

Immunoglobulin M (IgM) antibodies are the major and first class of antibodies found in the primary immune response and they are used to diagnose acute exposure to antigens (Boes, 2000). They are formed early in B-cell development (Schroeder and Cavacini, 2010) and they are found in the circulation principally as a pentamer structure (Ehrenstein and Notley, 2010).

IgM antibodies are more polyreactive than other isotypes, thus IgM-bearing B cells tend to respond quickly to antigens (Schroeder and Cavacini, 2010). IgM antibodies have an important function in the first line defense against invading pathogens and in tissue homeostasis because it regulates the removal of cellular remnants (Binder, 2010). Many studies have found a role for IgM antibodies in the protection against many viral, bacterial, fungal and parasitic infections (Ehrenstein and Notley, 2010).

1.2.3.1.2.2 Immunoglobulin G

Immunoglobulin G (IgG) is the predominant class of antibody found in the body and it constitutes about 75% of the antibodies of the normal healthy person (Schroeder and Cavacini, 2010; Guyton and Hall, 2006). It has the basic structure of antibodies and it is the major antibody in blood (Staines *et al*., 1994).

It has the longest serum half-life of all antibody classes and it is also the most extensively studied class of antibodies (Schroeder and Cavacini, 2010). IgG antibodies are associated with polysaccharide and protein antigens (Pituch-Noworolska and Błaut-Szlosarczyk, 2010). IgG antibodies directly contribute to an immune response, including protection of extravascular compartments from microorganisms and their toxins and neutralization of toxins and viruses

(Schroeder and Cavacini, 2010 and Staines *et al*., 1994). Due to its presence at the highest concentration of all antibodies, IgG is the most importance antibody in most antibody-mediated immune responses (Staines *et al*., 1994).

1.2.3.1.2.3 Immunoglobulin E

Immunoglobulin E (IgE) is a trace protein and normally forms about less than 0.001% of total serum antibodies (Anupama *et al*., 2005). IgE has a very short half-life and it is found in the blood at very low concentrations that are much lower than any other antibody. This is because some part of the circulating amount of IgE is continually removed and destroyed in endosomes (Platts-Mills, 2001). IgE is biologically active and it binds to high-affinity receptors on the surface of mast cells and basophils, so that these cells may be extremely sensitive to allergens even when the concentration of IgE in the blood is very low. In addition, the expression of the high-affinity receptors is upregulated during allergen-induced rhinitis, probably by IgE itself. Thus, the concentration of circulating IgE does not represent its true activity. Also the allergic immune response is comparatively selective, where IgE antibodies are specific to a chosen group of antigens and not for a wide range (Platts-Mills, 2001).

IgE antibodies are associated with hypersensitivity, allergic reactions, parasitic worm infections (Schroeder and Cavacini, 2010) and bronchial asthma in which the degrees of inflammation and the subsequent severity of airway obstruction are proportional to the serum IgE levels (Anupama *et al*., 2005).

1.2.3.1.2.4 Immunoglobulin A

The most abundant antibody isotype on most mucosal surfaces is secretory immunoglobulin A (SIgA), which is a polypeptide complex that consists of two

IgA monomers, the connecting J chain, and the secretory component (Snoeck *et al.*, 2006).

The internal surfaces of the gastrointestinal, respiratory, and genitourinary tracts represent major sites of potential attack by invading microorganisms. IgA is the principal antibody isotype that is found in the secretions of these mucosal surfaces and it acts as an important first line of defence. IgA antibody mediates a variety of protective functions by interaction with certain receptors and immune mediators. The importance of this protection is proved by the fact that some pathogens have evolved mechanisms to face IgA-mediated defence, providing an opportunity for extensive invasion (Woof and Kerr, 2006).

This abundance of IgA production in the intestinal mucosa depends on colonization with environmental microbes and is induced by the presence of commensal intestinal microbes. IgA can also act as a neutralizing antibody to pathogens and exotoxins (Macpherson *et al.*, 2008).

1.2.3.1.2.5 Immunoglobulin D

Immunoglobulin D (IgD) antibody is found at very low levels in the serum and it has a short serum half-life due to sensitivity of the molecule to proteolysis. Because it is not known to participate in the major antibody effector mechanisms, the function of circulating IgD is unclear. Circulating IgD can react with specific bacterial proteins independently of the variable regions of the antibody. These bacterial proteins bind to the constant regions of the IgD, which results in the stimulation and activation of B-cells (Schroeder and Cavacini, 2010).

Although the functions of IgD are not certain, it is found bound to the membranes of many B lymphocytes, and several functions have been suggested

(Vladutiu, 2000) such as it influences lymphocyte functions and has some role in allergic reactions (Staines *et al*., 1994). There are many conditions that lead to an increase (such as smoking and autoimmune diseases) or decrease (such as after radiotherapy) in the serum IgD levels but they have no practical values (Vladutiu, 2000).

IgD is co-expressed with IgM on the surface of most mature B cells before antigenic stimulation and it acts as a transmembrane antigen receptor. Secreted IgD has functions in blood, mucosal secretions and on the surface of innate immune effector cells such as basophils (Chen and Cerutti, 2011).

1.2.3.2 Cells of the myeloid series

1.2.3.2.1 Leukocytes

The leukocytes, or white blood cells, are the mobile units of the immune system and they are produced partially in the bone marrow (granulocytes and monocytes and a few lymphocytes) and partially in the lymph tissue (lymphocytes and plasma cells). After formation, they travel through the blood to different parts of the body (Guyton and Hall, 2006).

The role of the white blood cells is to transport to the site of infection and inflammation to provide a rapid and potent protection against infectious agents (Guyton and Hall, 2006). Leukocytes contain a nucleus and cytoplasm and they are divided into five subsets: neutrophil, eosinophil, basophil, monocyte and lymphocyte (Hiremath *et al.,* 2010). The neutrophil, eosinophil and basophil are called granulocytes because they contain granules in their cytoplasm.

1.2.3.2.1.1 Neutrophils

The most abundant leukocytes in humans are neutrophils and they are essential to innate immunity against invading pathogens (Kobayashi and DeLeo, 2009). The important functions of neutrophils are phagocytosis and direct killing of invading microorganisms via the production of oxygen intermediates and the release of lytic enzymes stored in their granules. Neutrophils contribute to the initiation of inflammation, which is an essential step in the launching of immunity, by promoting tissue injury and they also have an immunoregulatory role during microbial infection via the secretion of cytokines and chemokines (McFarlane *et al*., 2008).

There is much evidence that show the participation of neutrophils in allergy in general, especially in asthma. Neutrophil protease enzymes (lastase, cathepsin G, and proteinase-3) may increase airway inflammation in asthma by the activation of eosinophils to produce superoxide and neutrophilic cytokines and chemokines (Monteseirín, 2009).

1.2.3.2.1.2 Eosinophils

Eosinophils normally represent a small percent of circulating leukocytes (Behm and Ovington, 2000). Eosinophils are formed in the bone marrow and are released at a low rate into the blood stream. They have in their granules and lipid bodies potent cytotoxic and proinflammatory agents, and they express receptors and also produce a large variety of immunologically important molecules (Behm and Ovington, 2000).

The granules of eosinophils produce a series of cationic toxins which are able to kill many pathogens, including helminths, protozoa, bacteria, and other

cells (Gleich, 1993). Eosinophils have roles in host protection against parasites, in homeostatic function, including developmental biology and innate and adaptive immunities and also have a role in disease processes, including infections, asthma, and gastrointestinal disorders (Rothenberg and Hogan, 2006).

1.2.3.2.1.3 Basophils

Basophils are the least abundant leucocytes and they represent only a small percentage (less than 0.5%) of circulating blood cells under steady state conditions (Min *et al*., 2011). Their source of origin is the stem cells of the bone marrow where they complete their differentiation and development before transferring to the bloodstream (Arock *et al*., 2002). They expand rapidly in the bone marrow as a result of infections and pathogens and they are transferred by the blood to the sites of inflammation (Min *et al*., 2011).

Basophils have a short lifespan of about 60 hours during steady state conditions when the cells are under equilibrium and the concentrations of substances in and out the cells are not changed. After a helminth infection, they are quickly mobilized and can be efficiently entered into lymphoid and peripheral tissues where they exert their effective immune response (Voehringer, 2009). Basophils are known to have distinct and effective roles in allergic hypersensitivity reactions (Sullivan and Locksley, 2009).

1.2.3.2.1.4 Monocytes

Monocytes are circulating blood cells that represent about 10% of white blood cells in humans and they originate and develop in the bone marrow from stem cells. They are released to the bloodstream as non-dividing cells and their

half-life is around three days in humans and one day in mice (Yona and Jung, 2010).

Blood monocytes migrate during inflammation from blood to lymphoid and non-lymphoid tissues due to tissue-derived signals that may caused by infection or tissue damage (Woollard and Geissmann, 2010). The inflammatory monocytes differentiate into macrophages at the site of the inflammatory lesion of the infected tissue (Gordon and Taylor, 2005).

When the immune system activates macrophages, they become more powerful phagocytes than neutrophils, and they will be able to phagocytize approximately 100 bacteria and engulf much larger molecules, even whole red blood cells or malarial parasites, whereas neutrophils are not able to phagocytize molecules much larger than bacteria. After the digestion of the foreign agent, macrophages can remove the remnant products and often survive and function for many months (Guyton and Hall, 2006).

Tissue macrophages have different roles in the maintenance of tissue homeostasis by the clearance of senescent cells, tissue remodeling, and repairing and resolving the inflammatory tissues (Yona and Jung, 2010).

1.2.3.2.2 Thrombocytes (Platelets)

The platelet is small disc-shaped, partially refractile, colorless, containing many organelles but has no nucleus and has many roles (Berger and Toronto, 1970). The normal number of platelets in the circulation of human beings is in the range of 150,000 to 350,000/mm^3 (Berger and Toronto, 1970). They are formed from megakaryocytes, which are very large cells of the hematopoietic series in the

bone marrow (Guyton and Hall, 2006). Then, the cytoplasm of the megakaryocyte subdivides into platelets containing various organelles (Berger and Toronto, 1970).

Blood platelets are very active players in antimicrobial host defense and the promotion of inflammation and tissue repair, and they also participate in the hemostasis process (Klinger and Jelkmann, 2002). Platelets are very important in normal hemostasis to stop blood loss that results from vascular injury by adhering to sites of injury and attracting other platelets and blood cells to cause blood clotting (Spencer and Becker, 1997).

1.2.3.2.3 Erythrocytes (Red blood cells)

The most abundant cell type in blood is the erythrocyte. Erythrocytes are oval in shape and are red in color, which is due to the respiratory globin pigment know as hemoglobin, which is the most abundant protein in these cells (Morera and MacKenzie, 2011).

The important roles of erythrocytes are the transport of oxygen and carbon dioxide and in gas exchange. They also have a role in homeostasis, protecting against oxidative damage and regulating blood flow distribution in skeletal muscle. Human erythrocytes may play a role in modulating T cell survival and proliferation by enhancing cytokine production and induction of the interleukin-2 receptor (IL2R) (Morera and MacKenzie, 2011).

1.2.3.3 Cytokines

Cytokines are a group of secreted polypeptides, which mediate inflammation and they can be divided into two groups: those induced in acute inflammation and those involved in chronic inflammation (Feghali and Wright, 1997). One kind of

cytokines are the interleukins, which are a group of signaling molecules involved in communication between cells and they are formed and secreted by various types of cells, especially by cells of the immune system (Rose-John *et al*., 1992). Some interleukins are involved in acute inflammation, such as interlukin-1 and interlukin-6, and some others are involved in chronic inflammation, such as interlukin-3 and interlukin-10 (Feghali and Wright, 1997).

1.2.3.3.1 Interleukin-6

Interleukin-6 (IL-6) is a multifunctional cytokine which plays an important role in host defense because it has a wide range of immune and hematopoietic activities and it has an ability to induce the acute inflammatory response (Simpson *et al*., 1997). It also has a central role as a differentiation and growth factor of hematopoietic precursor cells, B cells, T cells, keratinocytes, neuronal cells, osteoclasts, and endothelial cells (Peters *et al*., 1996).

Substantial evidence indicates that IL-6 has pathological roles in various disease conditions, like inflammatory, autoimmune and malignant diseases (Kishimoto, 2010). Production of high concentrations of IL-6, which are noticed in patients with cardiac myxoma, synovial tissues of the joints or multiple lymph node swellings, can explain the inflammatory symptoms of the patients. The overproduction of IL-6 leads to the induction of different inflammatory diseases, such as acne and arthritis, and the de-regulation of IL-6 production is implicated in causing different diseases, including plasmacytoma/myeloma and some chronic inflammatory proliferative diseases (Kishimoto, 2010).

1.3 Lipid profile

1.3.1 Cholesterol

Cholesterol is a sterol substance which is a precursor of other body steroids and it is found in all body tissues, blood, bile, and fats (Guyton and Hall, 2006). There are two sources of cholesterol: exogenous cholesterol from the diet that is absorbed in the small intestine (Lewis, 1959) and endogenous cholesterol, which is formed in the body by the liver (Guyton and Hall, 2006). Also, all other cells of the body form at least some cholesterol, which enters in the formation of membranous structures (Guyton and Hall, 2006). Cholesterol is transported in lipoproteins and there are two types of cholesterol according to the lipoproteins: 'bad cholesterol' which transported in low density lipoproteins (LDL) from the liver to the tissues and 'good cholesterol' which is transported in high density lipoproteins (HDL) from the tissues to the liver (Whitney and Rolfes, 2005).

Cholesterol is an important molecule and it has many functions. It serves as a basic membrane component, a cofactor for signaling molecules, a source of steroid hormones formation (Pfrieger, 2003), a protective antioxidant, and it is essential for transmitting nervous impulses, especially at the level of the synapse (Colpo, 2005). Cholesterol is also important for the synthesis of bile acids, which are responsible for the absorption of fats, and it is essential in the production of vitamin D upon exposure to sunlight (Colpo, 2005). Increasing levels of cholesterol in the blood can lead to harmful effects because it accumulates in artery walls and causes atherosclerosis, which is responsible for heart attacks and strokes (Whitney and Rolfes, 2005).

1.3.2 Triglycerides

Triglycerides (TG), or triacylglycerols (TAG), are the most common form of fat in the diet and the major storage form of fat in the body. They consist of one molecule of glycerol connected to three fatty acids (Whitney and Rolfes, 2005). The triglycerides provide the body with energy needed for the different metabolic processes and they share this function equally with the carbohydrates. Some lipids, including cholesterol, phospholipids, and small amounts of triglycerides, enter in the formation of all cell membranes of the body and to achieve other cellular functions (Guyton and Hall, 2006).

Hypertriglyceridemia, which is an elevation of the triglycerides levels, is a common dyslipidemic feature associated with type 2 diabetes and prediabetic states (Tirosh *et al*., 2008). The fasting level of triglycerides ($\geq$ 150 mg/dl, or $\geq$ 1.70 mmol/l) is one of five accepted criteria (the others are high blood cholesterol, high blood LDL, low blood HDL and hypertension) for considering individuals at high risk for cardiovascular disease and type 2 diabetes and some evidence suggests that fasting triglyceride levels can aid in predicting future type 2 diabetes (Tirosh *et al*., 2008).

1.3.3 Low density lipoprotein

Lipoproteins are small particles consisting of triglycerides, cholesterol, phospholipids, and protein. Their primary function is to transport their lipid components in the blood, and especially they transport phospholipids and cholesterol from the liver to the peripheral tissues or from the tissues back to the liver. Low density lipoproteins are derived from lipoproteins by the removal of

almost all the triglycerides, leaving a high concentration of cholesterol and a moderately high concentration of phospholipids (Guyton and Hall, 2006).

Increasing serum LDL levels is a major risk factor of atherosclerosis and coronary heart disease (Ahmadi *et al.,* 2008). The oxidation of LDL makes it more atherogenic (Parthasarathy *et al*., 1992). After the vascular endothelium damage occurs, circulating monocytes and lipids, especially LDL, accumulate at the site of injury. Then, monocytes pass the endothelium and enter the intima of the vessel wall and there they become macrophages, which ingest and oxidize the accumulated lipoproteins giving the macrophages a foam form. These macrophage foam cells accumulate on the blood vessel to form a visible fatty plaque which causes atheroscelorosis (Guyton and Hall, 2006). Oxidation of LDL begins with the extraction of hydrogen from polyunsaturated fatty acids. Dietary fatty acids influence the fatty acid composition of LDL and cell membranes. Thus, the amount and type of fat in the diet may increase the oxidative damage of LDL and cells (Reaven and Witztum, 1996).

1.3.4 High density lipoprotein

High-density lipoprotein (HDL) is a type of lipoprotein that is composed of a high concentration of protein (about 50%) but much smaller concentrations of cholesterol and phospholipids when compared with LDL (Guyton and Hall, 2006). HDL is also the major lipoprotein which transfers cholesterol from different cells to the liver for excretion and catabolism by hepatocytes, which secrete cholesterol into the bile, and they also convert it to the primary bile acids (Miller, 1990).

In addition to cholesterol removal, other potentially antiatherogenic properties of HDL include inhibition of LDL oxidation, prevention of vascular

endothelial inflammation, promotion of endothelial nitric oxide production, promotion of prostacyclin bioavailability, and inhibition of platelet accumulation and coagulation in the vascular endothelium (Rader, 2006).

1.4 The relationship between stress and the immune system

There is extensive research on the effect of psychological stress on immunity and many models are utilized to investigate the impact of stress on immune function, such as bereavement, marital discord, care giving for a relative with a chronic disease, living with a cancer diagnosis, and academic exams stress (Agarwal and Marshall, 2001).

Academic examinations are a subject of stress research because they are predictable, standardized, and they are an example of real-life stressors. Academic examinations are associated with changes in mental and physical health including increased anxiety, increased negative mood, and changes in salivary pH, hormone levels, immune function and wound healing (Stowell, 2003). Studies suggest that the stress of academic examinations can have a significant effect on a student's well-being. Although academic examinations have been used in stress research since 1914, there has not been adequate discussion and explanation about the methodological and statistical issues associated with them (Stowell, 2003).

Studies on human and animal models showed that there are interactions between the central nervous system (CNS), endocrine, and immune systems and the impacts of various stressors on immunity. These studies demonstrate that psychological stressors have the ability to change the immune response, which leads to biological consequences such as impaired wound healing and an increased susceptibility to infectious diseases (Yang and Glaser, 2002). Clinical observations

have suggested that exposure to psychosocial stress can result in immune-related disorders such as viral infections, chronic autoimmune diseases and tumors. Studies published in the last two decades in the field of psychoneuroimmunology (PNI) have demonstrated that acute and chronic psychological stress can induce changes in innate and adaptive immune responses (Kemeny and Schedlowski, 2007).

Studies in the field of psychoneuroimmunology, which focuses on the interactions among the central nervous system (CNS), the endocrine system and the immune system, and the impact of these interactions on health (Padgett and Glaser, 2003), have shown that stress can result in the dysregulation of the immune system (Yang and Glaser, 2002). The connections between the neuroendocrine system and immune system provide a regulatory system required for health, but which in stress can lead to an imbalance of physiology of the body and enhanced-susceptibility to infection and inflammatory or autoimmune disease (Padgett and Glaser, 2003).

A wealth of clinical and pre-clinical research indicates that psychological stress suppresses the innate and adaptive immunities, which can ultimately affect disease onset and progression, inhibit wound healing, and increase the progression of cancer (Dhabhar**,** 2008 and Connor, 2008). Stress may result in cardiovascular risk and other conditions such as psoriasis and rheumatoid arthritis (Steptoe *et al*., 2007). On the other hand, there is evidence demonstrating that in some cases stress can promote immunity which can affect the progression of autoimmune disease (Connor, 2008). In general, psychological stress affects the immune system and predicts the susceptibility to infectious diseases (Marsland *et al*., 2002). Also, there are observations that suggest that stress may have bidirectional effects on

immunity, where it is immunosuppressive in some instances (as explained below) and immunoenhancing in others. Stress responses can be suppressive or enhancing of the immunity depending on the duration and intensity of the stressor (Dhabhar**,** 2008).

In contrast to chronic (long-term) stress that suppresses and dysregulates innate and acquired immunity, acute (short term) stress exerts a number of characteristic effects on the human cellular immune system which can promote the immunity (Dhabhar *et al*., 2010 and Atanackovic *et al*., 2006). Acute stress enhances innate immunity and suppresses acquired immunity (Kimura *et al*., 2005 and Hussain, 2010). Chronic stress may cause physical, behavioral and neuropsychological effects, such as anxiety, depression, cognitive dysfunction, cardiovascular diseases, metabolic disorders, such as obesity, and sleep disorders such as insomnia or excessive daytime sleepiness (Chrousos, 2009).

Segerstrom and Miller (2004) performed a meta-analysis on more than 300 empirical articles describing a relationship between psychological stress and parameters of the immune system in human participants. They found that acute stressors were associated with upregulation of some parameters of natural immunity and downregulation of some functions of specific immunity. Acute stress (such as exams) tended to suppress cellular immunity while preserving humoral immunity, while chronic stressors were associated with suppression of both cellular and humoral immunity.

1.4.1 The relationship between stress and blood cells

Acute stress may lead to changes in the numbers and percentages of white blood cells and the stress of intense exercise induces increases in the

concentrations of neutrophils, monocytes and lymphocytes in the blood (Pruett, 2003). In a study done by Segal *et al*. (2006) on twenty-five medical students, blood samples were collected from students immediately before an examination to analyze lymphocyte subpopulation counts, immunoglobulins (IgM, IgG and IgA) and phagocytic activity in neutrophils and monocytes. Another blood sample was collected from the same students in non-stressful situations and the results of the two blood samples were compared. The results showed no significant differences for any of the parameters analyzed. The researchers concluded that the immunologic evaluation of medical school students did not demonstrate an alteration in the lymphocyte subpopulation count, the phagocytotic response by monocytes, nor the phagocytosis digestion phase by neutrophils during acute stress (Segal *et al*., 2006).

The effect of stress reported in studies on fish, amphibians, reptiles, birds, mice, rats, rabbits, foxes, horses, non-human primates, and humans is a reduction in white blood cell numbers. In the first few minutes of stress, leukocyte numbers are increased but when the stressor is continued, they start to decrease and remain low for the duration of the stressor. Finally, if the stressor is terminated, leukocyte numbers are returned to pre-stress levels within a few hours after cessation of stress. The cessation of stress results in increased numbers of granulocytes, which do not return to pre-stress levels as rapidly as do lymphocyte and monocyte numbers. Red blood cell numbers, and hemoglobin, and hematocrit concentrations do not show any changes. Studies on rodents have shown that stress causes changes in the white blood cell numbers, which are characterized by a significant decrease in the numbers and percentages of lymphocytes and monocytes and by an increase in the numbers and percentages of neutrophils. In contrast to animal

studies, human studies have shown that stress can increase rather than decrease blood leukocyte numbers (Dhabhar, 2002; Dhabhar, 2008).

In the study by Kondo and Morimoto in 1996 to determine whether acute mental stress affects the composition of blood cells or components of the immune system, they determined blood cell counts and leukocyte differential counts, and examined lymphocyte subsets, before, during, and after 10 minutes of mental arithmetic in healthy female students. They found that during mental stress the absolute numbers of leukocytes and lymphocytes increased significantly but erythrocyte counts and hemoglobin concentrations remained unchanged during and after mental stress.

A study done by Patterson *et al*. (1995) examined the effects of psychological stress on hemoconcentration in women. Hematologic and hemodynamic variables were evaluated in women before and after 3 minutes of psychological stress (speech task). The results showed significant increase in hematocrit, hemoglobin levels, red and white blood cell counts. Bhatti and Shaikh (2007) carried a study on changes induced by physiological stress (moderate exercise) in haematological parameters of 88 healthy male and female students. Estimation of haemoglobin, total white blood cells count, differential white blood cells counts, erythrocyte sedimentation rate, and blood pressure were carried out before and after standard exercise (30 minutes jogging). They concluded that physiological stress leads to significant increases in total white blood cell counts in both male and female students.

Jern *et al*. (1989) investigated the effects of emotional stress on haematological parameters. The study was carried out on 29 healthy,

normotensive, non-smoking males aged 20–34 years and blood samples were collected before, during and after 10 minutes of mental arithmetic. There were significant increases in peripheral blood cells counts, haemoglobin concentration, and haematocrit in response to mental stress. The relative increments of leucocyte (8%) and platelet (3.5%) counts were significantly higher than the increase in haemoglobin concentration (2%). The increase in erythrocyte count, haemoglobin concentration, and haematocrit showed significant positive correlations with heart rate reactivity. The study concluded that emotional stress causes an increase in leucocyte and platelet counts.

Marita *et al*., in 2012, studied the effect of noise exposure as a stressor on biological and biochemical parameters. The study was done on newly trained tailors, who were exposed to acute noise stress for 7 hours per day. Their blood cell parameters were determined on the 8th and 24th day. The researchers observed significantly increased total leukocyte and platelet counts on the 24th day while there were no changes in red blood cell (RBC) counts and hemoglobin (Hb) concentrations.

A study by Mantur and Murthy (2010) to estimate white blood cell counts in both the absence and presence of examination stress and to compare pre-examination results to the results taken during exams on both male and female students. Results showed significantly higher numbers of all the subsets of leukocytes (lymphocytes, neutrophils, monocytes, basophils and eosinophils) during the examination period.

Wei *et al*., in 2008, studied the characteristic effects of psychological stress on serum iron and erythropoiesis in rats. Results showed that femoral bone marrow

iron was significantly decreased, serum iron and hemoglobin were highly significantly decreased, and RBC counts were significantly decreased.

1.4.2 The relationship between stress and Antibodies

The study done by Segal *et al*. (2006) on twenty-five medical students, described above, concluded that there was no alteration in the IgA, IgM and IgG serum levels during acute stress. Another study done by Silberman *et al*. (2003) on female mice, found that the production of IgG was enhanced after acute stress and impaired in chronic stress.

The effect of exposure to emotional stress on humoral immune function in adult male Wistar rats after injection with ovalbumin was studied by Shao *et al*. (2003). Emotional stress was induced by randomly giving empty water bottles to rats trained to drink water at specific times. The results showed that emotional stress induced a decrease in spleen weight and specific IgG antibody levels, and increased levels of epinephrine, norepinephrine and corticosterone. Thus, they concluded that emotional stress can change immune function, and the sympathetic nervous system may be involved in this immunomodulation.

Ursin *et al*. (1984) studied the relationships between two types of stress, chronic and acute, and plasma immunoglobulins. They found significant correlations between personality factors and immunoglobulins in the chronic stress group (all females), but not in the acute stress group (all males). For the chronic stress group, personality traits correlated negatively with concentrations of IgA and IgG, while IgM correlated positively. They concluded that psychological factors, at least chronic stress, correlate with immune processes and they supported the concept of the importance for psychological factors in immunological function.

Vassend and Halvorsen, in 1987, studied the effects of examination stress on serum concentrations of immunoglobulins in undergraduate students. The levels of antibodies were measured during, before and after examinations, and a control group comprised students not taking an examination. The results showed a small but significant decrease in IgM during stress and no other significant effects on IgA, IgG and IgE levels. They demonstrated a significant relationship between IgM concentrations and psychological variables reflecting acute or chronic psychological load, while there were no significant correlations between the psychological variables and IgA, IgG or IgE. Also the study found, immunoglobulin levels were not significantly correlated with serum cortisol concentration.

Another study was done by Jemmott and Magloire (1988) on the relation between academic stress and social support, and salivary concentrations of secretory immunoglobulin A (S-IgA). They assayed saliva samples collected from 15 healthy undergraduates five days before their final examination, during their examination, and 14 days after their last final exam for S-IgA concentrations. The students rated the university's general psychological climate as being more stressful during the exam period compared with the two other periods. Students who reported more adequate social support at the preexam period had consistently higher S-IgA levels than did their peers reporting less adequate social support. They concluded that social support enhances health outcomes irrespective of whether or not the individual is exposed to stressful experiences.

Murphy *et al*. (2010) carried a study on immune function and academic pressure before and during a midterm examination period. Undergraduate students were asked to complete a measure of global stress, the perceived stress scale (PSS-

10), and to indicate their current level of perceived stress. Students showed increased salivary cortisol concentrations and also reported greater acute perceived stress during the examination period compared to the non-examination period. Additional analyses showed a non-significant increase in the level of S-IgA from the non-examination period to the examination period. Specific pressure variables that appeared to contribute to stress regulation during the examination week included the amount of time spent studying and concern about the impact of examinations in the future. By demonstrating measures of chronic examination stress, these findings provide new insight into the complex relationship between examination stress, cortisol, and immune functioning.

In 1987, Scanlan *et al*. examined the effect of different rearing conditions and psychological stress on antibodies levels in rhesus monkey infants. Nursery monkey infants were subjected to social separations from peer groups at the age of 6 months. Before and after the first and fourth weeks of separation, plasma samples were collected and tested for IgG and IgM levels. The researchers detected small but significant decreases in IgG and IgM levels after four days of separation, and especially on the fourth week.

Maes *et al*. (1997) examined the effects of academic examination stress on serum IgA, IgG, and IgM levels in university students a few weeks before and after a difficult academic examination, and one day before a difficult academic examination. The students completed the Perceived Stress Scale (PSS) and they were divided into two groups, high- and low-stress groups, according to their PSS score. They found that academic examination stress induced significant increases in serum IgG and IgM in students with high-stress, but not in these with low-stress. The stress-induced changes in serum IgA concentrations were significantly

higher in students with high-stress than in those with low-stress. The stress-induced changes in serum IgA and IgM were normalized a few weeks after the stress condition, whereas IgG showed a trend toward normalization. Thus, the researchers concluded that there are significant positive relationships between the stress-induced changes measured by the PSS and serum IgA, IgG and IgM and they suggested that psychological stress was accompanied by changes in the secretion of serum immunoglobulins.

Endresen *et al*. (1987) studied nurses in a busy hospital ward and they found significant relationships between the plasma levels of antibodies and work-related stress, anxiety, and cognitive defense strategies. Their results indicated that immunological parameters may be used as an indicator of psychological stress, but the relationships were complex.

1.4.3 The relationship between stress and Interleukin-6

Kiecolt-Glaser *et al*. (2003) assessed the relationship between chronic stress and the proinflammatory cytokine IL-6 production. Their study was carried out on female and male older adults undergoing a chronic stressor (caregiving for a spouse with dementia) and another group not undergoing stress (non-caregivers). The caregivers' average rate of increase of IL-6 measured across 6 years was about four times as large as that of non-caregivers. A study was done by Steptoe *et al.* (2001) on the influence of acute mental stress on cardiovascular responses and concentrations of inflammatory cytokines. Blood IL-6 and saliva free cortisol were measured. The IL-6 concentration increased at 2 hours after the stress tasks, while no changes in cardiovascular variables or cytokine concentrations were observed

in the control subjects. This study concluded that inflammatory cytokines responded to acute mental stress.

In 2003, Kunz-Ebrecht *et al*. studied the individual differences in cortisol responses and their associations with proinflammatory cytokines, such as IL-6, cardiovascular activity, and mental health in healthy middle-aged subjects. They found that plasma IL-6 was higher in the cortisol non-responder group and they showed lower heart rate variability than the cortisol responders. The cortisol responder group experienced more subjective stress during the tasks and reported more impaired mental health than the non-responders. The study concluded that individual differences in neuroendocrine stress responsivity, by measuring if the subjects' cortisol concentrations increased or not, may have an effect on IL-6, and that both high and low cortisol stress responsiveness have potentially adverse effects.

Cohen *et al*. (1999) assessed the role of psychological stress in the expression of illness among infected subjects and they tested IL-6 production as a pathway linking stress to illness. The study was carried out on 55 subjects, who were experimentally infected with influenza A virus. The researchers found that higher psychological stress assessed before the viral infection was associated with greater symptom scores, greater mucus weights, and higher IL-6 nasal lavage concentrations in response to infection which was consistent with increases in IL-6 occurring in response to tissue damage associated with illness symptoms. It was concluded that psychological stress predicted a greater expression of illness and an increased production of IL-6 in response to an upper respiratory infection.

A study was done by Starkweather (2007) to determine the influence of the individual's level of stress and mood on proinflammatory cytokines. In the study, older adults (60 to 90 years old) who engage in regular physical activity were compared to non-exercisers. The participants in the exercise group reported significant improvements in stress and mood and they also had a significant decrease in serum IL-6.

1.5 The relationship between stress and cortisol

A study on university medical students (mean age of 21 years) was done by Shamsdin *et al*. (2010) to determine the effect of exam stress on serum cortisol levels. The study showed that exam stress resulted in a significant increase in cortisol levels. In another study (Weekes *et al*., 2006) on undergraduate students undergoing examination stress, researchers observed no significant correlations between elevations in psychological measures of stress and elevations in cortisol levels. Thus, no evidence was found to suggest a relationship between psychological and hormonal levels of stress. The findings of the study suggest the need to better define and consider the implications of both the specific measures of stress being used and individual differences in the subject samples in psychoendocrine studies.

Sadeghi *et al*. (2007) studied the effects of practical academic exams on serum ACTH and cortisol concentrations in normal and anxious students based on their scores on the anxiety standard questionnaire. The results showed that the stress of examinations significantly increased the levels of serum ACTH and cortisol in both normal and anxious student with no differences between the two

groups. Thus, the study concluded that examination stress can increase stress hormones.

Taylor *et al*. (2006) carried a study on older depressed and non-depressed patients at risk of cardiovascular disease (CVD) to compare their cortisol levels under stress. Results showed that there were no differences in baseline salivary cortisol levels or diurnal cortisol slopes, but depressed subjects showed significantly lower cortisol levels during the stress test and less cortisol response to stress. The researchers concluded that older depressed subjects with elevated risk for CVD exhibited a hypocortisol response to acute stress and this impaired cortisol response might lead to chronic inflammation and, in other ways, increase CVD risk. Burke *et al*. (2005) examined the association between depression and cortisol responses to psychological stressors. The study was applied on clinically depressed and non-depressed men and women with a mean age of 40 years. The results showed that depressed and non-depressed individuals had similar baseline and stress cortisol levels, but depressed patients had much higher cortisol levels during the recovery period than the non-depressed individuals.

Cruess *et al*. (2000) studied the effects of a cognitive-behavioral stress management (CBSM) group intervention on serum cortisol levels in women being treated for stage I or II breast cancer. Cortisol was measured before and after the state of the intervention. Intervention participants showed reduced serum cortisol levels, whereas control subjects experienced no change. The study demonstrated that stress management can influence physiological parameters such as cortisol among women with early stage breast cancer.

A study was done by Baig *et al*. (2006) to assess and correlate serum cortisol levels and self-perceived work-related stress among medical doctors working in emergency departments compared to doctors from non-emergency departments. Serum cortisol levels were measured in the morning and in the evening and two questionnaires were used to assess stress levels. Evening cortisol of emergency doctors was higher than non-emergency doctors and the difference between morning and evening cortisol was marginally significant. Evening serum cortisol concentration was significantly associated with the stress questionnaire. The study concluded that evening serum cortisol levels significantly correlated with work overload, however, there was marginally significant correlation between morning and evening serum cortisol difference and the study suggests that emergency physicians perceive more stress than non-emergency physicians.

Selimuzzaman *et al*. (2007) studied the effects of surgical stress on serum levels of cortisol in male patients (ages 18 and 45) undergoing surgical treatment to find out any differences in hormonal response between elective and emergency surgical procedures. The mean serum cortisol concentration in elective surgical cases was almost similar to that of healthy controls, but for the emergency surgical cases, a significantly increased mean cortisol level was observed. The study concluded that surgical intervention causes an increase in serum cortisol which was more marked in emergency cases.

1.6 The relationship between stress and the lipid profile

A study was done on healthy males by Patterson *et al*. (1993) to examine the effects of mental stress on serum cholesterol, triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL). Blood samples were

collected and levels of cholesterol, triglycerides, HDL, and LDL were estimated during a resting baseline, challenging mental arithmetic, and recovery. Results showed significant increases in cholesterol, triglycerides, LDL, and HDL levels during the mental arithmetic period and the study demonstrated that changes in lipid levels during stress resulted from rapid and substantial decrease in plasma volume that was caused by acute psychological stress. Patterson *et al*. (1995) examined the effects of psychological stress on hemoconcentration of blood cells and lipids in healthy women. Blood samples were collected before and after a speech task to assess the lipid profile. The results showed significant increases in total cholesterol, triglycerides, high density lipoprotein cholesterol and low density lipoprotein cholesterol during stress. The study demonstrated that acute stress can lead to most stress-induced changes in lipids.

In 2009, Qureshi *et al*. studied the changes in blood lipids, blood cortisol haemodynamic parameters during psychological stress in randomly selected males during stress and non-stress periods. The researchers found a significant increase in cortisol, systolic and diastolic blood pressures and heart rate during the stress period with $p < 0.001$ for each parameter but different blood lipids levels (cholesterol, LDL, HDL and TG) were detected with different significant levels.

Wattoo *et al*. in (2010) carried a study to evaluate environmental, psychological and physiological stresses in female nurses and housewives (ages 25-45 years) and to correlate them with their serum total cholesterol, HDL, LDL and triglycerides levels. The study showed that housewives had higher levels of total cholesterol, LDL and triglycerides and lower level HDL compared to nurses. Environmental, psychological and physiological stresses were significantly higher in housewives as compared to the nurses. The study demonstrated that there were

significant relationships between serum lipids levels and the three types of stresses.

Nayanatara *et al*. (2012) demonstrated the effect of chronic stress on some selected physiological, biochemical and lipid parameters in immobilization stressed and non-stressed rats. Immobilization stress is used to induce both psychological and physical stress. The results showed a significant increase in cholesterol, triglycerides and LDL in the stressed group when compared to the non stressed group but there were no significant changes in HDL levels. The researchers concluded that chronic immobilization stress causes significant alterations in the physiological, biochemical and lipid parameters.

Another study was done by Adekunle (2011) on healthy male and female students to investigate the relationship between academic stress and selected traditional markers of cardiovascular disorder such as lipids and lipoprotein profile and apoproteins. Results showed significant increases in total cholesterol, triglycerides, Apo A and B while there was a reduction in mean concentration of HDL during the academic stress period when compared with when there was no academic activity. These results suggested that stress may affect plasma lipids and lipoproteins. The relationship between examination stress to serum lipid profile was also studied by Bijlani *et al*. (1986). The study was done on male medical students before, near and after examinations and on control volunteers. As compared to pre-exam levels, total serum cholesterol, LDL and HDL in the medical students were increased significantly near exams, while control subjects did not show any significant change in the serum lipid profile. Further serial measurements revealed that examination-related changes were transient. The study

found that the response to examination stress may be related to the enhanced utilization of cholesterol in the adrenal cortex for steroidogenesis.

Researchers (Wattoo *et al*., 2007) studied the effects of stress on serum total cholesterol, HDL, and LDL and triglycerides levels among female health visitors and housewives between ages 25-40 years. Results of the study indicated that environmental, psychological and physiological stresses were significantly higher in housewives as compared to the health visitors. The levels of total cholesterol, LDL and triglycerides were higher in housewives than the health visitors and the level of HDL was lower in housewives as compared to the health visitors. The study concluded that the levels of total cholesterol, LDL and triglycerides increase but HDL levels decrease with the increase in the level of stress.

In 2004, Bacon *et al*. examined the effects of exercise and mental stress on lipid and immune reactions in patients with suspected coronary artery disease. Results showed that levels of total cholesterol, triglycerides, HDL and LDL increased with exercise, whereas levels of cholesterol and LDL increased with mental stress. This study provides evidence that exercise and mental stress lead to increases in lipid in patients with coronary artery disease.

The effects of short-term (mental) psychological stress on serum lipid levels, hemoconcentration and plasma viscosity in young adults was investigated by Muldoon *et al*. (1995). Results showed that the stressed subjects had significant increases in LDL, and HDL levels as compared to control (unstressed) subjects. The study provided evidence that exposure to short-term mental stress caused increases in serum lipid concentrations. The effect of psychological stress on plasma lipids was studied by McCann *et al*. (1996) in female and male law

students at the beginning of a term and before final exams. Perceived stress, perceived workload, and cortisol increased before examinations. Both LDL and apolipoprotein B (apo B) increased in men and women while HDL decreased in women only. Changes in cortisol and changes in LDL and apo B were associated, suggesting a neuroendocrine component to the effects. These results suggest that episodic, stressful situations may lead to potentially atherogenic changes in lipid and lipoprotein concentrations.

Maia *et al*. (2008) studied serum lipid composition in a sample of Brazilian police officers, with and without Post-Traumatic Stress Disorder (PTSD), regularly exposed to potentially traumatic situations. Results demonstrated that officers with PTSD exhibited significantly higher serum total cholesterol, LDL and triglycerides levels than those without PTSD. The researchers concluded that the association between PTSD and abnormal serum lipid profile and a tendency to exhibit higher BMI may lead to an increased risk for developing metabolic syndrome, a condition that by itself could account for many of the most serious PTSD-related physical health problems.

1.7 Aim of the study

The aim of this study is to determine the effect of acute stress of final examinations on the immune system in healthy female university students of King Abdulaziz University. This is especially important since this effect is not fully understood, nor is there a consensus whether it is a suppressive or enhancing effect. This will be achieved by collecting and analyzing blood samples taken from subjects at a stress-free time and during the examinations weeks, and by the use of a questionnaire, filled in by the subjects, designed to assess the level of stress that

they are experiencing. The results of the questionnaire and the levels of some measured stress hormones (cortisol and adrenalin) and the lipid profile will help to determine the level of stress for each subject, which is then correlated to the immune system parameters. The results should help establish whether acute stress affects the immune response, and if so, is it an enhancing or depressing effect.

CHAPTER II

Materials and Methods

Chapter II

Materials and Methods

2.1 Subjects and Anthropometric Measurements

This study was carried out using a total of 41 healthy randomly chosen Saudi female students (19-26 years old) from King Abdulaziz University, Jeddah, Saudi Arabia. None of the subjects were pregnant, on medications, or menstruating at the time of blood collection. Also none of the subjects had any major diseases, such as diabetes, high blood pressure, blood diseases, anemia, allergies, immunological diseases and genetic diseases according to their answers on the questionnaire about health state.

The parameters were assessed in the group of students on two occasions: at a stressful period (during the final examinations weeks) and at a stress-free period (beginning of the term). All subjects filled in a consent form, a general health form, and the Perceived Stress Questionnaire (PSQ) which is an approved questionnaire designed to assess the level of stress that a subject is experiencing (Levenstein *et al.*, 1993).

The weight of each subject was measured at the first and second blood collections, using a new regular household scale, while the height was measured

using a fabric measuring tape at the first blood collection only. The waist and hips circumferences were measured, using a fabric measuring tape, at both blood collections. The waist was measured at the navel and the hips were measured at the fullest point.

To assess the body weight of the subjects, three different methods were used. These were the body mass index (BMI), the waist-to-hip ratio (WHR) and the waist circumference (WC).

BMI was calculated by dividing the student's weight by her height squared. Based on this calculated BMI, the students were divided into five groups, or categories, as follows: an underweight group (BMI below 18.9), a healthy group (BMI between 18.9–24.9), an overweight group (BMI from 25-29.9), an obese group (BMI in the range 30-40) and finally a morbid obesity group (BMI above 40).

The waist-to-hip ratio (WHR) was calculated for each subject. The subjects were classified into three risk groups: the low risk (WHR $\leq$ 0.80), moderate risk (0.81 $\leq$ WHR $\leq$ 0.85), and high risk (WHR > 0.85) groups.

The waist circumference (WC) classifies subjects into two groups: high risk group for waist circumferences greater than 88 cm and low risk group for waist circumferences lower than 88 cm (Rolfes *et al.* 2006).

2.1.1 Categorizations of Subjects

Subjects (n = 41) of the study were assessed at a stressful time (exam period) and a non-stressful time or condition (regular lectures, non-exam period).

The measured parameters were compared in three different ways leading to the three sections of the results. The sections are:

Section one: Results of the parameters for the two times (or cases) are compared.

Section two: Results of the two cases, stress case and no stress case, are classified into three categories (low, intermediate, and high stress groups) of perceived stress based on the total score obtained for each subject using the Perceived Stress Questionnaire (PSQ) (Levenstein *et al.*, 1993), which is widely used to asses stress.

Section three: Results of the two cases (stress case and no stress case) are classified into two categories (low and high stress groups) of perceived stress based on the total score obtained for each subject using the Perceived Stress Questionnaire (PSQ) (Levenstein *et al.*, 1993).

2.1.2 Blood Collection

Blood samples were collected into two types of vacutainer tubes. Eethylene diamine tetra-acetic acid (EDTA) vacutainer tubes were used for whole blood for differential complete blood counts (CBC), while plain vacutainer tubes were used for blood serum for the determination of all other parameters.

Blood in plain tubes was centrifuged at 3,000 rpm for 10 minutes to separate the serum from the blood clot. Subsequently the separated serum was transferred into eppendorf tubes using a micropipette, and finally stored at -80°C until the tests were performed. EDTA vacutainer tubes were stored in a container containing ice for about 4 to 5 hours and then they were transferred to the

Hematology Lab at King Abdulaziz University Hospital, Jeddah, Saudi Arabia for CBC analysis.

2.1.3 Parametric Analysis

2.1.3.1 Differential Complete Blood Count

The differential complete blood count (CBC) for each blood sample was done on a Coulter LH 700 Series (Beckman Coulter Inc., Brea, CA, USA) at the King Abdulaziz University Hospital, Jeddah, Saudi Arabia.

Principle:

The coulter method counts and measures the size of cells by detecting and measuring changes in electrical resistance when cells in a conductive liquid pass through a small aperture. Total red cells, white cells, and platelets counts were determined by this method. Also the counts of white blood cells subtypes (neutrophils, lymphocytes, monocytes, eosinophils and basophils) were determined.

The Coulter LH 700 Series along with the control and calibrator CBC reagents, CBC cleaning agent, and CBC analysis reagents were used according to the manufacturer's instructions.

2.1.3.2 Determination of Serum Triglycerides Concentration

The concentrations of serum triglycerides were determined using an *in vitro* Flex Reagent Cartridge Kit (Siemens Healthcare Diagnostics Ltd., Camberley, UK) and the analysis was performed on the Dimension Clinical

Chemistry System RXL max, at the King Abdulaziz University Hospital, Jeddah, Saudi Arabia.

Principle:

The determination of serum triglycerides concentration is based on an enzymatic procedure, in which a combination of enzymes is employed. The samples were incubated with lipoprotein lipase (LPL) which converts triglycerides to free glycerol and fatty acids as shown below.

$$\text{Triglycerides} \xrightarrow{\text{LPL}} \text{Glycerol + Fatty Acids}$$

Glycerol kinase (GK) catalyzes the phosphorylation of glycerol by adenosine-5-triphosphate (ATP) to glycerol-3-phosphate:

$$\text{Glycerol + ATP} \xrightarrow{\text{GK}} \text{Glycerol-3-phosphate + ADP}$$

Glycerol-3-phosphate–oxidase (GPO) then oxidizes glycerol-3-phosphate to dihydroxyacetone phosphate and hydrogen peroxide (H_2O_2):

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{GPO}} \text{Dihydroxyacetone phosphate} + H_2O_2$$

Finally, quinonemine was formed from H_2O_2, aminoantipyrine and 4-chlorophenol by the catalytic action of peroxidase (POD):

$$2\ H_2O_2 + \text{Aminoantipyrine + 4-chlorophenol} \xrightarrow{\text{POD}} \text{quinoneimine + HCl} + 4\ H_2O$$

The change in absorbance due to the formation of quinoneimine is directly proportional to the total amount of glycerol and its precursors in the sample and was measured using a biochromatic (510, 700 nm) endpoint technique.

2.1.3.3 Determination of Total Serum Cholesterol Concentration

The concentrations of serum cholesterol were determined using an *in vitro* Flex Reagent Cartridge Kit (Siemens Healthcare Diagnostics Ltd., Camberley, UK) and the analysis was performed on the Dimension Clinical Chemistry System RXL max, at the King Abdulaziz University Hospital, Jeddah, Saudi Arabia.

Principle:

The hydrolysis of cholesterol esters was catalyzed by cholesterol esterase (CE) to produce free cholesterol:

$$\text{Cholesterol esterase} \xrightarrow{CE} \text{Cholesterol} + \text{Fatty Acids}$$

The produced free cholesterol, along with preexisting free cholesterol, was oxidized in a reaction catalyzed by cholesterol oxidase (CO) to form cholest-4-ene3-one and hydrogen peroxide:

$$\text{Cholesterol} + O_2 \xrightarrow{CO} \text{Cholest-4-ene-3-one} + H_2O_2$$

N, N-diethylaniline-HCL/4-aminoantipyrine (DEA-HCL/AAP) was oxidized by hydrogen peroxide (that was formed due the presence of horseradish peroxidase (HPO)) to produce a chromophore that absorbs at 540 nm:

$$2\ H_2O_2 + \text{DEA.HCl/AAP} \xrightarrow{HPO} \text{Dihydroxyacetone phosphate} + H_2O_2$$

The absorbance due to the oxidized DEA-HCL/AAP is directly proportional to the total cholesterol concentration and was measured using a polychromatic (452, 540, and 700 nm) endpoint technique.

2.1.3.4 Determination of Serum HDL-Cholesterol Concentration

Serum high-density lipoprotein-cholesterol (HDL-C) concentrations were determined using an *in vitro* Flex Reagent Cartridge Kit (Siemens Healthcare Diagnostics Ltd., Camberley, UK) and the analysis was performed on the Dimension Clinical Chemistry System RXL max, at the King Abdulaziz University Hospital, Jeddah, Saudi Arabia.

Principle:

The method for HDL-cholesterol estimation is a two reagents format that depends on the properties of a unique detergent. The method is based on accelerating the reaction of cholesterol oxidase (CO) with non-HDL unesterified cholesterol and dissolving HDL selectively using a specific detergent provided by the kit. In the first step, non-HDL unesterified cholesterol was subjected to an enzymatic reaction and the peroxide generated was consumed by a peroxidase reaction with N, N-bis(4-sulphobutyl)-m-toluidine-disodium salt (DSBmT) yielding a colorless product by the following reaction:

$$\text{HDL, LDL, and VLDL, chylomicrons} \xrightarrow[\text{+DSBmT +Peroxidase}]{\text{Accelerator +CO}} \text{Non-reactive LDL, and VLDL,Chylomicrons}$$

The second step was using a detergent capable of solubilizing HDL specifically, cholesterol esterase (CE) and chromagenic coupler to develop color for the quantitative determination of HDL-C. The absorbance was read at 600 nm and 700 nm wavelengths. This method might be referred to as the Accelerator Selective Detergent methodology.

$$\text{HDL} \xrightarrow{\text{HDL Specific Detergent}} \text{HDL disrupted}$$

$$\text{HDL cholesterol} \xrightarrow{\text{Chol-esterase + Chol-oxidase}} \text{Cholestenone} + H_2O_2$$

$$H_2O_2 + \text{DSBmT} + \text{4-AAP} \xrightarrow{\text{Peroxidase}} \text{Color development}$$

2.1.3.5 Determination of Serum LDL-Cholesterol

Serum low-density lipoprotein-cholesterol (LDL-C) concentrations were determined using an *in vitro* Flex Reagent Cartridge Kit (Siemens Healthcare Diagnostics Ltd., Camberley, UK) and the analysis was performed on the Dimension Clinical Chemistry System RXL max, at the King Abdulaziz University Hospital, Jeddah, Saudi Arabia.

Principle:

The method uses a two reagent format and it depends on the properties of detergent 1 that is provided by the kit, which solubilizes only non LDL particles in the following reaction.

$$\text{Nonsoluble LDL-C, VLDL-C, and HDL-C, Chylomicrons}$$
$$\xrightarrow{\text{Detergent 1}} \text{Soluble non-LDL-C (VLDL-C, and HDL-C, Chylomicrons)}$$

The LDL-cholesterol released was consumed by cholesterol esterase and cholesterol oxidase in a non-color forming reaction:

$$\text{Soluble non-LDL-C} \xrightarrow{\text{Chol-Esterase + Chol-oxidase}} \text{Non-color forming}$$

The remaining LDL particles were solubilized by detergent 2. The soluble LDL-C was then oxidized by the reaction of cholesterol esterase and cholesterol oxidase forming cholestenone and hydrogen peroxide (H_2O_2):

$$\text{Nonsoluble LDL-C} \xrightarrow{Detergent\ 2} \text{Soluble LDL-C}$$

$$\text{Soluble LDL-C} + O_2 \xrightarrow{\text{Chol-Esterase} + \text{Chol-oxidase}} \text{Colestenone} + H_2O$$

The color was produced by the enzymatic action of peroxidase on H_2O_2 in the presence of N, N-bis(4-sulfobutyl)-m-toluidine, disodium salt (DSBmT) and 4-aminoantipyrine (4-AA). Finally, the color was measured using a bichromatic (540, 700 nm) endpoint technique, and the color produced is directly proportional to the amount of LDL-C present in the sample:

$$H_2O_2 + \text{DSBmT} + \text{4-AA} \xrightarrow{Peroxidase} \text{Color development}$$

2.1.3.6 Quantitative Determination of Total Serum Cortisol Concentration

The quantitative determination of total serum cortisol concentration was done using a Monobind Cortisol ELISA Kit (Monobind Inc., Lake Forest, CA, USA) and the analysis was performed on a Microplate Reader ELX800 (Biotek Instruments, Winooski, VT, USA) at the King Fahad Center for Medical Research.

The cortisol ELISA kit uses a specific monoclonal anti-cortisol antibody and contains reagents and materials as follows:

human serum references, cortisol enzyme reagent, cortisol conjugate buffer, cortisol biotin reagent, streptavidin coated plate, wash solution, substrate A, substrate B and stop solution.

Principle:

A specific human monoclonal anti-cortisol is used by the Monobind cortisol ELISA kit. The construction of a standard curve to determine concentrations was permitted by the employment of several serum references of known cortisol concentrations.

The essential reagents required for the enzyme immunoassay include antibody, enzyme-antigen conjugate and the native antigen. A competition reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of antibody binding sites. A stimulation reaction between the biotin attached to the antibody and the streptavidin immobilized on the microwell occurs. That affected the separation of the antibody bound fraction after decantation or aspiration of the reagents.

The enzyme activity in the antibody-bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentrations, a dose response curve was generated from which the antigen (cortisol) concentration can be determined.

2.1.3.7 Qualitative Determination of Serum IgG and IgM Concentrations

The qualitative determination of serum immunoglobulins IgG, and IgM concentrations was done by using IgG-ClC and IgM-ClC ELISA kits (DRG International, Inc. Mountainside, NJ, USA) and the analysis was performed on a Microplate Reader ELX800 (Biotek Instruments, Winooski, VT, USA) at the King Fahad Center for Medical Research. Each kit contains the following reagents and materials:

incubation buffer, conjugate (peroxidase-conjugate anti-IgG or peroxidase-conjugate anti-IgM), conjugate buffer, TMB (Tetramethyl benzidine) substrate, coated microplate and stop solution**.**

Principle:

An immunoenzymatic colorimetric method was used for the qualitative determination of serum IgG and IgM concentrations.

The C3-fixing circulating immune complexes (CIC) that are present in the samples were first blocked by an anti–C3 antibody immobilized on a microplate. To quantify IgG-CIC complexes a specific peroxidase anti-IgG antibody and the enzyme substrate H_2O_2-TMB were then employed. Similarly, to quantify IgM-CIC complexes, a specific peroxidase anti-IgM antibody and the enzyme substrate H_2O_2-TMB were then added.

The amounts of formed products, which were measured by reading absorbances at wavelength 450 nm, are proportional to the levels of solid-phase IgG-CIC and IgM-CIC complexes. The levels of IgG-CIC and IgM-CIC complexes are equal to the serum levels of IgG and IgM, respectively.

The absorbance (OD) and the mean OD of controls were needed for the calculation of standard deviation (SD):

$$sd = \frac{OD_{sample} - Mean1_{Controls}}{SD1_{Controls}}$$

Values of sd less than 2.0 are considered to be negative for significant levels of the antibody, while values of sd greater than or equal to 2.0 are considered to be positive for significant levels of the antibody.

2.1.3.8 Quantitative Determination of Serum IgA, IgE and IgD Concentrations

The quantitative determination of serum IgA, IgE and IgD concentrations was based on solid phase enzyme-linked immunosorbent assays. The kits used were: Total Human IgE Assay Kit, and Total Human IgA Assay Kit (both Diagnostic Automation, Inc., Calabasas, LA, USA); and Total Human IgD Assay Kit (ALPCO Diagnostics, Salem, NH, USA). The analyses were performed on a Microplate Reader ELX800 (Biotek Instruments, Winooski, VT, USA) at the King Fahad Center for Medical Research.

Each kit contains the following reagents and materials: antibody-coated microtiter wells (with anti-IgA, anti-IgE, or anti-IgD), reference standards, buffer, enzyme conjugate reagent, TMB substrate, stop solution and wash buffer concentrate.

Principle:

The assay system utilizes one (anti-IgA, anti-IgE or anti-IgD) antibody for solid phase (microtiter wells) immobilization and another (horseradish peroxidase-conjugated anti-IgA, anti-IgE or anti-IgD) antibody in the antibody-enzyme conjugate solution. The serum was added to the microtiter wells and it was incubated with the buffer. Then the antibodies present in the serum were combined with the anti-antibodies on the well. The wells were then washed to remove any

residual serum, and subsequently the specific (IgA, IgE or IgD) horseradish peroxidase conjugated antibody was added. The conjugate was bound immunologically to the antibody in the well, resulting in the antibody molecules being sandwiched between the solid phase and enzyme-linked antibodies. After incubation at room temperature, the wells were washed with water to remove unbound labeled antibodies. A solution of tetramethylbenzidine (TMB) was added and incubated for 20 min, resulting in the development of color. The color development was stopped with the addition of stop solution and it was measured spectrophotometrically at 450 nm. The concentration of the serum antibody is directly proportional to the color intensity of the test serum.

2.1.3.9 Quantitative Determination of Serum Interleukin-6 Concentration

The quantitative determination of serum interleukin 6 (IL6) concentrations was done by using the Human Interleukin-6 (Hu IL-6) ELISA Kit (ALPCO Diagnostics, Salem, NH, USA) and it was performed on Microplate Reader ELX800 (Biotek Instruments, Winooski, VT, USA) at the King Fahad Center for Medical Research.

The kit contains the following reagents and materials:

Hu IL-6 standard, standard diluent buffer, Hu IL-6 antibody-coated wells, Hu IL-6 biotin conjugate, streptavidin-peroxidase or horseradish peroxidase (HRP), streptavidin-peroxidase (HRP) diluent, wash buffer, stabilized chromogen Tetramethylbenzidine (TMB), stop solution and plate covers.

Principle:

A monoclonal antibody specific for Hu IL-6 has been coated onto the wells of the microtiter plate. Samples, standards of known Hu IL-6 concentrations, and control specimens were pipetted into the wells, followed by the addition of a biotinylated monoclonal second antibody. During this incubation, the Hu IL-6 antigen binds simultaneously to the immobilized antibody on one site, and to the solution phase biotinylated antibody on a second site.

After removal of excess second antibody, streptavidin-peroxidase (HPR) was added and was bound to the biotinylated antibody. After a second incubation and washing to remove all the unbound enzyme, a substrate solution was added, which was acted upon by the bound enzyme to produce color. The intensity of this colored product is directly proportional to the concentration of Hu IL-6 present in the original specimen. The color was measured spectrophotometrically at 450 nm.

2.2 Statistical methods

All the reported data were recorded on a worksheet and the statistical program SPSS-V12 was used to obtain the descriptive and analytical statistics described below.

2.2.1 Descriptive statistics

The following statistical results were calculated for all parameters: Mean, Standard deviation (± SD), and Standard error of the mean (± SE).

2.2.2 Analytical statistics

After checking the normality and the homogeneity assumptions for the parameters of the two dependent samples, it was noted that the assumptions were satisfied and the paired sample t-test was used for comparing means between two samples before examination and during examinations.

After testing the normality and the homogeneity assumptions for the three groups and the two groups, it was noted that the assumptions were satisfied for some parameters and not satisfied for other parameters. The one way ANOVA test was used when the normality was satisfied to determine if any significant differences between groups averages exist and the Kruskal-Wallis test was used when the normality was not satisfied to find any significant differences between groups averages.

For the qualitative variables, when significant relationships were found between two variables, Chi-square test was used if the following assumption was satisfied (expected frequency for all cells greater than five). But for the qualitative variables in this research work, the assumption was not satisfied thus, Fisher's-test was used as appropriate test.

The general results for all tests based on the p-value are shown below:

$P \geq 0.05$ = Non Significant (NS)

$P < 0.05$ = Significant (S)

$P < 0.01$ = Highly Significant (HS)

CHAPTER III

Results

Chapter III

Results

3.1 Section one: Uncategorized comparisons of the two cases

In this section the results for the stress case (n = 41) were compared with those of the no stress case (n = 41). All results, unless noted otherwise, for this section were analyzed using the paired samples t-test and the mean, standard deviation (SD), standard error (SE) and P-value were determined for the parameters of each case.

3.1.1 The differential complete blood count test

The counts of the total white blood cells (WBCs) and their subtypes (neutrophils, eosinophils, basophils, lymphocytes and monocytes) were determined for the two cases, as shown in Table 3.1. Also, red blood cell and platelet counts along with the hemoglobin concentrations were determined for the two groups.

Using the paired samples t-test, the results showed a highly significant increase ($P < 0.01$) for the total white blood cells, neutrophil, and lymphocyte counts. Non-significant changes ($P > 0.05$) are obtained for monocytes,

TABLE 3.1 The differential CBC for uncategorized comparisons of the two cases using paired samples t-test.

Parameters	Non final examination period (n = 41)	Final examination period (n = 41)	P-value
	Mean ± SD (± SE)	Mean ± SD (± SE)	
WBC (10^3/µL)	6.59 ± 1.93 (± 0.30)	7.95 ± 1.80 (± 0.28)	0.0001 ^{HS}
Neutrophils (10^3/µL)	3.60 ± 1.39 (± 0.22)	4.60 ± 1.76 (± 0.27)	0.0001 ^{HS}
Lymphocytes (10^3/µL)	2.30 ± 0.74 (± 0.12)	2.65 ± 0.77 (± 0.12)	0.001 ^{HS}
Monocytes (10^3/µL)	0.50 ± 0.27 (± 0.04)	0.49 ± .21 (± 0.03)	0.959 ^{NS}
Eosinophils (10^3/µL)	0.16 ± 0.14 (± 0.02)	0.16 ± 0.13 (± 0.02)	0.881 ^{NS}
Basophils (10^3/µL)	0.02 ± 0.04 (± 0.01)	0.03 ± 0.05 (± 0.01)	0.200 ^{NS}
RBC (10^6/µL)	4.43 ± 0.60 (± 0.09)	4.63 ± 0.36 (± 0.06)	0.029 ^{S}
HGB (g/dL)	12.35 ± 0.31 (± 0.95)	12.56 ± 1.18 (± 0.18)	0.433 ^{NS}
PLT (10^3/µL)	285.15 ± 77.51 (± 12.11)	295.02 ± 70.45 (± 11.00)	0.276 ^{NS}

HS = highly significant at P-value < 0.01
S = significant at P-value < 0.05
NS = not significant at P-value ≥ 0.05

eosinophils, basophils, hemoglobin and platelets and a significant increase ($P < 0.05$) for the red blood cell counts for the stress case compared to the no stress case.

3.1.2 Serum levels of the lipid profile

As shown in Table 3.2, there are no significant changes ($P > 0.05$) in the cholesterol, triglycerides and high density lipoprotein (HDL) concentrations between the two cases, except for the low density lipoprotein (LDL), which is slightly (but not significantly, $P = 0.053$) higher in the stress group compared with the no stress group.

3.1.3 Serum levels of cortisol and IL-6

Table 3.3 shows a highly significant increase in the mean serum level of cortisol in the stress case as compared to the no stress case. No significant change in the serum levels of IL-6 is observed between the two groups.

3.1.4 Serum levels of IgE, IgA, IgD, IgM and IgG antibodies

There are no significant changes ($P > 0.05$) in the serum levels of IgE, IgA and IgD between the two cases, as shown in Table 3.4.

Since the results for the serum concentrations of the IgM and IgG antibodies are qualitative, Fisher's test is used for the analysis of the data. The results (Table 3.5) show that there was a significant increase ($P < 0.05$) in IgM concentration of the stress case as compared to the no stress case, while there is a highly significant decrease ($P < 0.01$) in IgG of the stress case as compared to the no stress case.

TABLE 3.2 Serum levels of the lipid profile for the uncategorized comparisons of the two cases using paired samples t-test.

Parameters (mmol/L)	Non final examination period (n = 41)	Final examination period (n = 41)	P-value
	Mean ± SD (± SE)	Mean ± SD (± SE)	
Cholesterol	4.23 ± 0.76 (± 0.12)	4.25 ± 0.71 (± 0.11)	0.729 NS
TG	0.70 ± 0.27 (± 0.04)	0.73 ± 0.33 (± 0.05)	0.357 NS
HDL	1.87 ± 0.36 (± 0.06)	1.89 ± 0.36(± 0.06)	0.489 NS
LDL	2.58 ± 0.69 (± 0.11)	2.69 ± 0.64 (± 0.10)	0.053 NS*

NS = not significant at P-value ≥ 0.05
* = tends to significance

TABLE 3.3 Serum levels of cortisol and IL-6 for the uncategorized comparisons of the two cases using paired samples t-test.

Parameters	Non final examination period (n = 41)	Final examination period (n = 41)	P-value
	Mean ± SD (± SE)	Mean ± SD (± SE)	
Cortisol (μg/dL)	8.54 ± 4.66 (± 0.73)	12.20 ± 4.62 (± 0.72)	0.001 ^{HS}
IL-6 (pg/mL)	3.30 ± 7.45 (± 1.24)	5.07 ± 15.42 (± 2.57)	0.197 ^{NS}

HS = high significant at P-value < 0.01
NS = not significant at P-value ≥ 0.05

TABLE 3.4 Serum levels of IgE, IgA and IgD for the uncategorized comparisons of the two cases using paired samples t-test.

Antibodies	Non final examination period (n = 41)	Final examination period (n = 41)	P-value
	Mean ± SD (± SE)	Mean ± SD (± SE)	
IgE (IU/mL)	164.29 ± 219.03 (± 34.21)	180.56 ± 212.43 (± 33.18)	0.504 ^{NS}
IgA (µg/dL)	4.19 ± 8.69 (± 1.36)	2.88 ± 10.04 (± 1.57)	0.535 ^{NS}
IgD (ng/mL)	47.75 ± 60.37 (± 10.67)	46.32 ± 40.78 (±7.21)	0.825 ^{NS}

NS = not significant at P-value ≥ 0.05

TABLE 3.5 Serum levels of IgM and IgG for the uncategorized comparisons of the two cases using Fisher's test.

Antibodies (mg/dL)	Non final examination period (n = 41)				Final examination period (n = 41)				P-value
	No. of sig.	% of sig.	No. of non sig.	% of non sig.	No. of sig.	% of sig.	No. of non sig.	% of non sig.	
IgM	3	7.3%	38	92.7%	5	12.2%	36	87.8%	0.035 [S]
IgG	8	19.5%	33	80.5%	7	17.1%	34	82.9%	0.001 [HS]

S = significant at P-value < 0.05 HS = high significant at P-value < 0.01

3.2 Section two: Categorized comparisons of the parameters (three categories)

The subjects were categorized using the Perceived Stress Questionnaire (PSQ) in each of the no examinations period and examinations period individually. Thus, the subjects were categorized twice producing the three categories for each of the cases. Categorization into the three groups was according to the total score of each subject in the questionnaire. Subjects with a total score between 30 and 64 were recognized as the low stress group, subjects with a total score between 65 and 85 were recognized as the intermediate stress group and, finally, subjects with a total score between 86 and 120 were recognized as part of the high stress group. Subsequently, the results of the three groups of the two cases were compared.

The examinations period had 41 subjects and this number of subjects was divided into three groups as follows: low stress group (n = 22, 53.7%), intermediate stress group (n = 13, 31.7%) and high stress group (n = 6, 14.6%). The no examinations period also had 41 subjects divided into three groups as follows: low stress group (n = 22, 53.7%), intermediate stress group (n = 17, 41.5%) and high stress case (n = 2, 4.9%).

For the subjects during the no exam period, the categorization was carried on the subjects during that period and the results for the parameters were compared with the results for the same subjects during the exam period, not considering the categorization obtained for the exam period. This comparison is termed part A in all involved tables. Similarly, for the subjects during the exam period, as done above, the categorization was carried on the subjects during that period and the parameters were compared with the results for the same subjects in the no exam period, again not considering the categorization there. This comparison is termed

part B in all involved tables. In both cases the examination period mean concentrations are compared with the no examination period (control) mean concentrations.

In this section of results, the paired samples t-test was used to determine the mean, standard deviation (SD), standard error (SE) and P-value for all the parameters of each of the groups (categories) within the two cases (periods).

3.2.1 The differential complete blood count test

The counts of the total white blood cells, and their subtypes (neutrophils, eosinophils, basophils, lymphocytes and monocytes) were determined for the three groups of the two cases, as shown in Table 3.6.a and Table 3.6.b. Also, red blood cell and platelet counts along with the hemoglobin concentrations were determined for the three groups of the two cases, as shown in Table 3.6.c.

Using the paired samples t-test, the results show a highly significant increase ($P < 0.01$) in white blood cells of the low and intermediate stress groups of the two cases, during no final examinations and during final examinations. For white blood cells of the high stress group during final examinations case there is a significant increase ($P < 0.05$) but during no final examinations there is no significant change ($P > 0.05$) but the difference tended to a significant increase ($P = 0.051$).

For neutrophils there is a significant increase ($P < 0.05$) in the mean counts of the low and intermediate stress groups of the two cases. As for the high stress group of the two cases there are no significant changes ($P > 0.05$) but the difference tended to significant increase ($P = 0.053$) during final examinations.

TABLE 3.6.a The differential complete blood count test results for the categorized (three groups) comparisons of the two cases using paired samples t-test.

Parameter	Group of stress level	Part A: Non final examination period (n = 41)		
		$Mean_1$ ± SD (± SE)	$Mean_2$ ± SD (± SE)	P-value
WBC (10^3/µL)	Low n = 22	6.24 ± 1.86 (± 0.40)	7.53 ± 1.86 (± 0.40)	0.005 HS
	Inter. n =17	7.22 ± 1.96 (± 0.47)	8.54 ± 1.69 (± 0.41)	0.002 HS
	High n = 2	5.00 ± 0.85 (± 0.60)	7.50 ± 1.13 (± 0.80)	0.051 NS*
Neutrophils (10^3/µL)	Low n = 22	3.38 ± 1.35 (± 0.29)	4.29 ± 1.87 (± 0.40)	0.014 S
	Inter. n = 17	3.98 ± 1.44 (± 0.35)	5.04 ± 1.64 (± 0.40)	0.014 S
	High n = 2	2.70 ± 0.71 (± 0.50)	4.35 ± 1.34 (± 0.95)	0.170 NS
Lymphocytes (10^3/µL)	Low n = 22	2.24 ± 0.68 (± 0.15)	2. 60 ± 0.57 (± 0.12)	0.014 S
	Inter. n =17	2.45 ± 0.83 (± 0.20)	2.74 ± 1.01 (± 0.24)	0.103 NS
	High n = 2	1.70 ± 0.14 (± 0.10)	2.40 ± 0.28 (± 0.20)	0.090 NS
Parameter	**Group of stress level**	**Part B: Final examination period (n = 41)**		
		$Mean_1$ ± SD (± SE)	**$Mean_2$ ± SD (± SE)**	**P-value**
WBC (10^3/µL)	Low n = 22	6.60 ± 1.54 (± 0.33)	7.50 ± 1.72 (± 0.37)	0.002 HS
	Inter. n = 13	6.64 ± 2.56 (± 0.71)	8.67 ± 1.88 (± 0.52)	0.007 HS
	High n = 6	6.42 ± 2.01 (± 0.82)	8.03 ± 1.62 (± 0.66)	0.039 S
Neutrophils (10^3/µL)	Low n = 22	3.50 ± 1.33 (± 0.28)	4.21 ± 1.86 (± 0.40)	0.011 S
	Inter. n = 13	3.79 ± 1.54 (± 0.43)	5.15 ± 1.46 (± 0.41)	0.037 S
	High n=6	3.53 ± 1.47 (± 0.60)	4.83 ± 1.87 (± 0.76)	0.053 NS*
Lymphocytes (10^3/µL)	Low n = 22	2.35 ± 0.61 (± 0.13)	2.61 ± 0.65 (± 0.14)	0.068 NS
	Inter. n = 13	2.25 ± 1.04 (± 0.29)	2.77 ± 1.07 (± 0.30)	0.024 S
	High n = 6	2.20 ± 0.47 (± 0.19)	2.52 ± 0.36 (± 0.15)	0.214 NS

HS = high significant at P-value < 0.01 S = significant at P-value < 0.05
NS = not significant at P-value ≥ 0.05 * = tends to significance
$Mean_1$= mean during non exams period $Mean_2$ = mean during exams period

TABLE 3.6.b The differential complete blood count test results for the categorized (three groups) comparisons of the two cases using paired samples t-test.

Parameter	Group of stress level	Part A: Non final examination period (n = 41)		
		$Mean_1$ ± SD (± SE)	$Mean_2$ ± SD (± SE)	P-value
Monocytes (10^3/µL)	Low n = 22	0.43 ± 0.16 (± 0.03)	0.45 ± 0.22 (± 0.05)	0.687 NS
	Inter. n = 17	0.59 ± 0.36 (± 0.09)	0.55 ± 0.21 (± 0.05)	0.634 NS
	High n = 2	0.40 ± 0.00 (± 0.00)	0.55 ± 0.07 (± 0.05)	0.205 NS
Eosinophils (10^3/µL)	Low n = 22	0.16 ± 0.14 (± 0.03)	0.16 ± 0.11 (± 0.02)	1.000 NS
	Inter. n = 17	0.17 ± 0.13 (± 0.03)	0.16 ± 0.15 (± 0.04)	0.826 NS
	High n = 2	0.15 ± 0.21 (± 0.15)	0.15 ± 0.21 (± 0.15)	No result
Basophils (10^3/µL)	Low n = 22	0.02 ± 0.04 (± 0.01)	0.03 ± 0.05 (± 0.01)	0.492 NS
	Inter. n = 17	0.02 ± 0.04 (± 0.01)	0.04 ± 0.05 (± 0.01)	0.431 NS
	High n = 2	0.00 ± 0.00 (± 0.00)	0.05 ± 0.07 (± 0.05)	0.500 NS
Parameter	**Group of stress level**	**Part B: Final examination period (n = 41)**		
		$Mean_1$ ± SD (± SE)	$Mean_2$ ± SD (± SE)	P-value
Monocytes (10^3/µL)	Low n = 22	0.53 ± 0.30 (± 0.06)	0.46 ± 0.20 (± 0.04)	0.305 NS
	Inter. n = 13	0.42 ± 0.24 (± 0.07)	0.56 ± 0.25 (± 0.07)	0.022 S
	High n = 6	0.52 ± 0.19 (± 0.08)	0.48 ± 0.15 (± 0.06)	0.732 NS
Eosinophils (10^3/µL)	Low n = 22	0.18 ± 0.16 (± 0.03)	0.18 ± 0.12 (± 0.03)	1.000 NS
	Inter. n = 13	0.14 ± 0.10 (± 0.03)	0.14 ± 0.15 (± 0.04)	1.000 NS
	High n = 6	0.15 ± 0.12 (± 0.05)	0.13 ± 0.10 (± 0.04)	0.695 NS
Basophils (10^3/µL)	Low n = 22	0.02 ± 0.04 (± 0.01)	0.02 ± 0.04 (± 0.01)	1.000 NS
	Inter. n = 13	0.01 ± 0.03 (± 0.01)	0.04 ± 0.05 (± 0.01)	0.040 S
	High n = 6	0.03 ± 0.05 (± 0.02)	0.05 ± 0.06 (± 0.02)	0.695 NS

S = significant at P-value < 0.05 NS = not significant at P-value ≥ 0.05

$Mean_1$= mean during non exams period $Mean_2$ = mean during exams period

TABLE 3.6.c The differential complete blood count test results for the categorized (three groups) comparisons of the two cases using paired samples t-test.

Parameter	Group of stress level	Part A: Non final examination period (n = 41)		
		$Mean_1$ ± SD (± SE)	$Mean_2$ ± SD (± SE)	P-value
RBC (10^6/µL)	Low n = 22	4.28 ± 0.74 (± 0.16)	4.57 ± 0.30 (± 0.06)	0.064 NS
	Inter. n = 17	4.62 ± 0.33 (± 0.08)	4.69 ± 0.45 (± 0.11)	0.255 NS
	High n = 2	4.54 ± 0.08 (± 0.06)	4.65 ± 0.20 (± 0.14)	0.405 NS
HGB (g/dL)	Low n = 22	12.13 ± 2.48 (± 0.53)	12.64 ± 1.18 (± 0.25)	0.295 NS
	Inter. n = 17	12.55 ± 1.12 (± 0.27)	12. 40 ± 1.25 (± 0.30)	0.002 HS
	High n = 2	13.15 ± 0. 50 (± 0.35)	13.10 ± 0.42 (± 0.30)	0.500 NS
PLT (10^3/µL)	Low n = 22	272.50 ± 69.85 (± 14.89)	288.14 ± 64.41 (± 13.73)	0.312 NS
	Inter. n = 17	307.47 ± 86.60 (± 21.00)	311.12 ± 77.88 (± 18.89)	0.706 NS
	High n = 2	234.50 ± 27.58 (± 19.50)	234.00 ± 38.18 (± 27.00)	0.958 NS
Parameter	**Group of stress level**	**Part B: Final examination period (n = 41)**		
		$Mean_1$ ± SD (± SE)	**$Mean_2$ ± SD (± SE)**	**P-value**
RBC (10^6/µL)	Low n = 22	4.48 ± 0.27 (± 0.06)	4.64 ± 0.36 (± 0.08)	0.000 HS
	Inter. n = 13	4.33 ± 1.02 (± 0.28)	4.57 ± 0.44 (± 0.12)	0.391 NS
	High n = 6	4.48 ± 0.16 (± 0.06)	4.71 ± 0.20 (± 0.08)	0.024 S
HGB (g/dL)	Low n = 22	12.57 ± 1.07 (± 0.23)	12.61 ± 1.15 (± 0.24)	0.664 NS
	Inter. n = 13	11.75 ± 3.16 (± 0.88)	12.32 ± 1.41 (± 0.39)	0.503 NS
	High n = 6	12.88 ± 0.42 (± 0.17)	12.93 ± 0.67 (± 0.28)	0.752 NS
PLT (10^3/µL)	Low n = 22	276.23 ± 62.49 (± 13.32)	285.82± 61.30 (± 13.07)	0.291 NS
	Inter. n = 13	307.77 ± 108.06 (± 29.97)	322.92 ± 87.26 (± 24.20)	0.545 NS
	High n = 6	268.83 ± 40.46 (±16.52)	268.33 ± 48.94 (±19.98)	0.949 NS

HS = high significant at P-value < 0.01
S = significant at P-value < 0.05
NS = not significant at P-value ≥ 0.05
$Mean_1$= mean during non exams period
$Mean_2$ = mean during exams period

Table 3.6.a also shows a highly significant increase ($P < 0.01$) in lymphocytes of the low stress group during the no final examinations and no significant changes ($P > 0.05$) during the final examinations. For lymphocytes of the intermediate stress group, there is a significant increase ($P < 0.05$) in lymphocytes of the final examinations case and no significant changes ($P > 0.05$) in lymphocytes of the no final examinations case. Finally, there are no significant changes ($P > 0.05$) in lymphocytes of the high stress group of the two cases.

Eosinophils of the three groups of the two cases (Table 3.6.b) shows no significant changes ($P > 0.05$) but there is no P-value for eosinophils of the high stress group of the no final examinations case due to the fact that the standard error for the two high stress groups is zero. Monocytes and basophils of the intermediate group of the final examinations case show a significant increase ($P < 0.05$) but for monocytes and basophils of the other groups of the two cases and of the intermediate group of no final examinations case there are no significant changes ($P > 0.05$).

There is a highly significant increase ($P < 0.01$) in the red blood cells (Table 3.6.c) of the low stress group of final examinations case and a significant increase ($P < 0.05$) in red blood cells of the high stress group of final examinations case. For other groups of the two cases there are no significant changes ($P > 0.05$) in red blood cells.

For hemoglobin there is a highly significant decrease ($P < 0.01$) in hemoglobin only for the intermediate group of no final examinations case, while there are no significant changes ($P > 0.05$) in hemoglobin for all other groups of the two cases.

The results also demonstrate that there are no significant changes (P > 0.05) in the platelets of the three groups of the two cases.

3.2.2 Serum lipid profile

Table 3.7.a and Table 3.7.b represent the results of the serum levels of the lipid profile by using the paired samples t-test. The results demonstrate that there are no significant changes (P > 0.05) between the two cases of the three groups for the serum levels of cholesterol, triglycerides (TG), low density lipoprotein (LDL), and low density lipoprotein (LDL).

3.2.3 The serum levels of cortisol and IL-6

Using the paired samples t-test, Table 3.8 demonstrates that there is a highly significant increase (P < 0.01) in cortisol concentration of the low stress concentrations of the low and intermediate stress group of no final examinations case. There are non significant changes (P > 0.05) in the cortisol concentrations group of the final examinations case and significant increases (P < 0.05) in the of the intermediate stress group of no final examinations case and of the high stress group of the two cases. Table 3.8 also shows that there are no significant changes (P > 0.05) in the IL-6 concentrations of the three stress level groups of the two cases.

TABLE 3.7.a The serum levels of lipid profile results for the categorized (three groups) comparisons of the two cases using paired samples t-test.

Parameter (mmol/L)	Group of stress level	Part A: Non final examination period (n = 41)		
		$Mean_1$ ± SD (± SE)	**$Mean_2$ ± SD (± SE)**	**P-value**
Cholesterol	Low n = 22	4.16 ± 0.81 (± 0.17)	4.14 ± 0.71 (± 0.15)	0.830 NS
	Inter. n = 17	4.33 ± 0.73 (± 0.18)	4.41 ± 0.73 (± 0.18)	0.455 NS
	High n = 2	4.20 ± 0.83 (± 0.59)	4.09 ± 0.35 (± 0.25)	0.918 NS
TG	Low n = 22	0.63 ± 0.19 (± 0.04)	0.62 ± 0.17 (± 0.04)	0.716 NS
	Inter. n = 17	0.76 ± 0.35 (± 0.09)	0.82 ± 0.45 (± 0.11)	0.180 NS
	High n = 2	0.79 ± 0.21 (± 0.15)	0.88 ± 0.03 (± 0.02)	0.586 NS
Parameter (mmol/L)	**Group of stress level**	**Part B: Final examination period (n = 41)**		
		$Mean_1$ ± SD (± SE)	**$Mean_2$ ± SD (± SE)**	**P-value**
Cholesterol	Low n = 22	4.11 ± 0.82 (± 0.18)	4.08 ± 0.72 (± 0.15)	0.654 NS
	Inter. n =13	4.37 ± 0.67 (± 0.19)	4.42 ± 0.58 (± 0.16)	0.695 NS
	High n = 6	4.37 ± 0.74 (± 0.30)	4.54 ± 0.842 (± 0.34)	0.594 NS
TG	Low n = 22	0.64 ± 0.24 (± 0.05)	0.63 ± 0.17 (± 0.04)	0.770 NS
	Inter. n = 13	0.73 ± 0.29 (± 0.08)	0.77 ± 0.37 (± 0.10)	0.660 NS
	High n = 6	0.78 ± 0.35 (± 0.14)	1.04 ± 0.54 (± 0.22)	0.082 NS

NS = not significant at P-value ≥ 0.05 $Mean_1$= mean during non exams period
$Mean_2$ = mean during exams period

TABLE 3.7.b The serum levels of the lipid profile for the categorized (three groups) comparisons of the two cases using paired samples t-test.

Parameter (mmol/L)	Group of stress level	Part A: Non final examination period (n = 41)		
		$Mean_1$ ± SD (± SE)	**$Mean_2$ ± SD (± SE)**	**P-value**
HDL	Low n = 22	1.92 ± 0.27 (± 0.06)	1.91 ± 0.28 (± 0.06)	0.775 NS
	Inter. n = 17	1.90 ± 0.46 (± 0.11)	1.87 ± 0.43 (± 0.11)	0.480 NS
	High n = 2	1.56 ± 0.37 (± 0.26)	1.56 ± 0.09 (± 0.06)	1.000 NS
LDL	Low n = 22	2.47 ± 0.67 (± 0.14)	2.56 ± 0.64 (± 0.14)	0.143 NS
	Inter. n = 17	2.70 ± 0.75 (± 0.18)	2.84 ± 0.66 (± 0.16)	0.162 NS
	High n = 2	2.79 ± 0.33 (± 0.23)	2.82 ± 0.50 (± 0.35)	0.967 NS
Parameter (mmol/L)	**Group of stress level**	**Part B: Final examination period (n = 41)**		
		$Mean_1$ ± SD (± SE)	**$Mean_2$ ± SD (± SE)**	**P-value**
HDL mmol/L	Low n = 22	1.85 ± 0.32 (± 0.07)	1.86 ± 0.32 (± 0.07)	0.765 NS
	Inter. n = 13	1.94 ± 0.34 (± 0.10)	1.86 ± 0.36 (± 0.10)	0.065 NS
	High n = 6	1.96 ± 0.54 (± 0.22)	1.97 ± 0.51 (± 0.21)	0.969 NS
LDL	Low n = 22	2.49 ± 0.69 (± 0.15)	2.56 ± 0.65 (± 0.14)	0.341 NS
	Inter. n = 13	2.71 ± 0.75 (± 0.21)	2.83 ± 0.61 (±0.17)	0.252 NS
	High n = 6	2.65 ± 0.60 (± 0.25)	2.89 ± 0.65 (±0.27)	0.248 NS

NS = not significant at P-value ≥ 0.05 $Mean_1$= mean during non exams period
$Mean_2$ = mean during exams period

TABLE 3.8 The serum levels of cortisol and IL-6 results of for the categorized (three groups) comparisons of the two cases using paired samples t-test.

Parameter	Group of stress level	Part A: Non final examination period (n = 41)		
		$Mean_1$ ± SD (± SE)	$Mean_2$ ± SD (± SE)	P-value
Cortisol (µg/dL)	Low n = 22	9.35 ± 5.40 (± 1.15)	13.16 ± 4.07 (± 0.87)	0.020 S
	Inter. n = 17	7.95 ± 3.60 (± 0.87)	11.72 ± 4.87 (± 1.18)	0.030 S
	High n = 2	4.70 ± 0.42 (± 0.30)	5.80 ± 4.52 (± 3.20)	0.806 NS
IL-6 (pg/mL)	Low n = 22	4.31 ± 10.20 (± 2.34)	7.59± 21.13 (± 4.85)	0.211 NS
	Inter. n = 17	2.13 ± 1.38 (± 0.36)	2.19 ± 1.40 (± 0.36)	0.749 NS
	High n = 2	2.35 ± 0.21 (± 0.15)	2.60 ± 1.13 (± 0.80)	0.766 NS
Parameter	**Group of stress level**	**Part B: Final examination period (n = 41)**		
		$Mean_1$ ± SD (± SE)	**$Mean_2$ ± SD (± SE)**	**P-value**
Cortisol (µg/dL)	Low n = 22	8.91 ± 3.74 (± 0.80)	12.65 ± 3.10 (± 0.85)	0.005 HS
	Inter. n = 13	9.46 ± 6.39 (± 1.77)	11.42 ± 3.72 (± 1.03)	0.364 NS
	High n = 6	5.20 ± 1.03 (± 0.42)	12.25 ± 8.24 (± 3.36)	0.090 NS
IL-6 (pg/mL)	Low n = 22	4.38 ± 10.53 (± 2.48)	7.18 ± 21.80 (± 5.14)	0.309 NS
	Inter. n = 13	2.21 ± 1.02 (± 0.28)	3.23 ± 2.53 (± 0.70)	0.139 NS
	High n = 6	2.18 ± 0.92 (± 0.41)	2.22 ± 0.93 (± 0.42)	0.953 NS

HS = high significant at P-value < 0.01
S = significant at P-value < 0.05
NS = not significant at P-value ≥ 0.05
$Mean_1$ = mean during non exams period
$Mean_2$ = mean during exams period

3.2.4 Serum levels of IgE, IgA, IgD, IgM and IgG antibodies

Table 3.9 shows that there are no significant changes ($P > 0.05$) observed in the serum levels of IgE, IgA and IgD for the three stress groups of the two cases.

Since the results for the serum concentrations of the IgM and IgG antibodies were qualitative, thus Fisher's test was used for the analysis of the data. The results (Table 3.10) show that there is a significant increase ($P < 0.05$) for IgM concentration of the intermediate stress level group of the no examinations period and no significant change for IgM concentration of the intermediate stress level group of examinations period. Also there are no significant changes for IgM concentration of the low stress level group of the two cases. A highly significant decrease ($P < 0.01$) is shown for IgG concentrations of the intermediate stress level group of the two cases, while there are no significant changes ($P > 0.05$) for IgG concentration of the low stress level of the two cases. There were no P-values for IgM and IgG concentrations of the high stress level of the two cases because the percentages of significant of IgM and IgG concentrations were constant.

TABLE 3.9 Serum levels of IgE, IgA and IgD for the categorized (three groups) comparisons of the two cases using paired samples t-test.

Parameter	Group of stress level	Part A: Non final examination period (n = 41)		
		$Mean_1$ ± SD (± SE)	$Mean_2$ ± SD (± SE)	P-value
IgE (IU/mL)	Low n = 22	193.16 ± 242.52 (± 51.71)	205.78 ± 214.96 (± 45.83)	0.757 NS
	Inter. n = 17	139.90 ± 197.88 (± 47.99)	154.78 ± 222.15 (± 53.88)	0.560 NS
	High n = 2	54.10 ± 67.57 (± 47.78)	122.31 ± 113.74(± 80.42)	0.689 NS
IgA (µg/dL)	Low n = 22	1.89 ± 3.72 (± 0.79)	4.74 ± 13.51 (± 2.88)	0.336 NS
	Inter. n = 17	5.41 ± 9.38 (± 2.28)	0.81 ± 1.44 (± 0.35)	0.057 NS
	High n = 2	19.13 ± 27.04 (± 19.12)	0.03 ± 0.02 (± 0.01)	0.500 NS
IgD (ng/mL)	Low n = 22	53.75 ± 70.26 (± 18.14)	41.98 ± 36.38 (± 9.39)	0.320 NS
	Inter. n = 17	46.17 ± 54.16 (± 13.98)	49.32 ± 44.75 (± 11.56)	0.859 NS
	High n = 2	14.59 ± 5.64 (± 3.99)	56.47 ± 66.00 (± 46.67)	0.373 NS
Parameter	**Group of stress level**	**Part B: Final examination period (n=41)**		
		$Mean_1$ ± SD (± SE)	**$Mean_2$ ± SD (± SE)**	**P-value**
IgE (IU/mL)	Low n = 22	159.73 ± 238.04 (± 50.75)	163.64 ± 218.61 (± 46.61)	0.917 NS
	Inter. n = 13	211.19 ±229.69 (± 63.71)	239.41 ± 241.59 (± 67.01)	0.518 NS
	High n = 6	79.39 ± 68.65 (±15.98)	115.13 ± 69.33 (± 28.30)	0.358 NS
IgA (µg/dL)	Low n = 22	2.80 ± 6.98 (±1.49)	4.69 ± 13.53 (± 2.88)	0.564 NS
	Inter. n = 13	4.23 ± 7.23 (± 2.01)	0.66 ± 0.74 (± 0.21)	0.109 NS
	High n = 6	9.24 ± 15.31 (± 6.25)	1.08 ± 2.33 (± 0.95)	0.244 NS
IgD (ng/mL)	Low n = 22	57.60 ± 77.69 (± 18.84)	48.65 ± 47.65 (± 11.56)	0.266 NS
	Inter. n = 13	43.28 ± 33.79 (±10.68)	45.33 ± 30.25 (± 9.57)	0.651 NS
	High n = 6	23.19 ± 17.80 (± 7.96)	40.38 ± 40.97 (± 18.32)	0.506 NS

NS = not significant at P-value ≥ 0.05
$Mean_1$= mean during non exams period
$Mean_2$ = mean during exams period

TABLE 3.10 Serum levels of IgM and IgG for the categorized comparisons (three groups) of the two cases using Fisher's test.

Parameters (mg/dL)	Group of stress level	Part A: Non final examination period (n = 41)					Part B: Final examination period (n = 41)				
		No. of sig.	% of sig.	No. of non sig.	% of non sig.	P-value	No. of sig.	% of sig.	No. of non sig.	% of non sig.	P-value
IgM	Low	1	2.4%	21	51.2%	0.909 NS	3	7.3%	19	46.3%	0.260 NS
	Inter.	2	4.9%	15	36.6%	0.022 S	2	4.9%	11	26.8	0.154 NS
	High	0	0%	2	4.9%	No result	0	0%	6	14.6%	No result
IgG	Low	4	9.8%	18	43.9%	0.338 NS	3	7.3%	19	46.3%	0.117 NS
	Inter.	4	9.8%	13	31.7%	0.000 HS	3	7.3%	10	24.4%	0.003 HS
	High	0	0%	2	4.9%	No result	1	2.4%	5	12.2%	No result

HS = high significant at P-value < 0.01 NS = not significant at P-value ≥ 0.05

3.2.5 Comparison of the average of the 3 groups of the two cases at normal and non-normal distributions for the parameters

In this part the average of the three (low, intermediate, and high) stress groups for each parameter of the two cases (final examinations and not final examinations) are compared. Two statistical analyses were used, the ANOVA test for the parameters with a normal distribution and the Kruskal-Wallis test for the parameters with a non-normal distribution.

3.2.5.1 Comparison of the average of the 3 groups of the two cases at normal and non-normal distributions for CBC

Table 3.11 represents the results of the average of white blood cells, neutrophils, lymphocytes, monocytes, eosinophils, basophils, red blood cells, platelets counts and hemoglobin concentrations for the three groups of two cases. The results show no significant changes ($P > 0.05$) between the two cases.

3.2.5.2 Comparison of the average of the 3 groups of the two cases at normal and non-normal distributions for lipid profile

Table 3.12 shows the results of the average of cholesterol, triglycerides (TG), high density lipoproteins (HDL) and low density lipoproteins (LDL) between the three groups of the two cases. The results show no significant changes ($P > 0.05$) in the lipid profile between the two cases.

3.2.5.3 Comparison of the average of the 3 groups of the two cases at normal and non-normal distributions for cortisol and IL-6

The results of the average of cortisol and IL-6 between the three groups of the two cases is shown in Table 3.13, and the results demonstrate

TABLE 3.11 Comparison of the average of the 3 groups of the two cases at normal and non-normal distributions for the differential complete blood count test.

Parameter	Part A: Non final examinations period (n = 41)				
	Type of test	Mean	SD	SE	P-value
WBC (10^3/µL)	ANOVA	6.585	1.932	0.302	0.145 NS
Neutrophils (10^3/µL)	ANOVA	3.595	1.387	0.217	0.274 NS
Lymphocytes (10^3/µL)	ANOVA	2.298	0.741	0.116	0.351 NS
Monocytes (10^3/µL)	Kruskal-Wallis	0.495	0.268	No result	0.430 NS
Eosinophils (10^3/µL)	Kruskal-Wallis	0.161	0.138	No result	0891 NS
Basophils (10^3/µL)	Kruskal-Wallis	0.020	0.040	No result	0.371 NS
RBC (10^6/µL)	Kruskal-Wallis	4.431	0.599	No result	0.839 NS
HGB (g/dL)	Kruskal-Wallis	12.354	1.951	No result	0.654 NS
PLT (10^3/µL)	ANOVA	285.146	77.511	12.105	0.246 NS
Parameter	**Part B: Final examinations period (n = 41)**				
	Type of test	**Mean**	**SD**	**SE**	**P-value**
WBC (10^3/µL)	ANOVA	7.946	1.798	0.281	0.176 NS
Neutrophils (10^3/µL)	ANOVA	4.600	1.756	0.274	0.304 NS
Lymphocytes (10^3/µL)	ANOVA	2.646	0.767	0.120	0.766 NS
Monocytes (10^3/µL)	ANOVA	0.493	0.211	0.033	0.358 NS
Eosinophils (10^3/µL)	Kruskal-Wallis	0.159	0.128	No result	0.395 NS
Basophils (10^3/µL)	Kruskal-Wallis	0.032	0.047	No result	0.373 NS
RBC (10^6/µL)	ANOVA	4.625	0.364	0.057	0.726 NS
HGB (g/dL)	ANOVA	12.561	1.176	0.184	0.561 NS
PLT (10^3/µL)	ANOVA	295.024	70.448	11.002	0.197 NS

NS = not significant at P-value ≥ 0.05

TABLE 3.12 Comparison of the average of the 3 groups of the two cases at normal and non-normal distributions for lipid profile.

Parameter (mmol/L)	Part A: Non final examinations period (n = 41)				
	Type of test	**Mean**	**SD**	**SE**	**P-value**
Cholesterol	ANOVA	4.230	0.759	0.119	0.789 [NS]
TG	Kruskal-Wallis	0.691	0.272	No result	0.470 [NS]
HDL	ANOVA	1.891	0.357	0.056	0.409 [NS]
LDL	ANOVA	2.582	0.691	0.108	0.546 [NS]
Parameter (mmol/L)	**Part B: Final examinations period (n = 41)**				
	Type of test	**Mean**	**SD**	**SE**	**P-value**
Cholesterol	ANOVA	4.252	0.705	0.110	0.222 [NS]
TG	Kruskal-Wallis	0.726	0.331	No result	0.107 [NS]
HDL	ANOVA	1.873	0.355	0.055	0.790 [NS]
LDL	ANOVA	2.691	0.642	0.100	0.354 [NS]

NS = not significant at P-value ≥ 0.05

TABLE 3.13 Comparison of the average of the 3 groups of the two cases at normal and non-normal distributions for cortisol and IL-6.

Parameter	Part A: Non final examinations period (n = 41)				
	Type of test	**Mean**	**SD**	**SE**	**P-value**
Cortisol (µg/dL)	Kruskal-Wallis	8.542	4.664	No result	0.042 [S]
IL-6 (pg/mL)	Kruskal-Wallis	3.224	7.164	No result	0.285 [NS]
Parameter	**Part B: final examinations period (n = 41)**				
	Type of test	**Mean**	**SD**	**SE**	**P-value**
Cortisol (µg/dL)	ANOVA	12.200	4.618	0.721	0.760 [NS]
IL-6 (pg/mL)	Kruskal-Wallis	4.883	15.017	No result	0.230 [NS]

NS = not significant at P-value ≥ 0.05 S = significant at P-value < 0.05

significant changes ($P < 0.05$) in the cortisol concentrations between low stress group, intermediate stress group and high stress group of the no final examinations case.

There are no significant differences ($P > 0.05$) in the cortisol concentrations of the three groups of the final examinations case. Also, the table shows that there is no significant differences ($P > 0.05$) in IL-6 of the three groups of the two cases.

3.2.5.4 Comparison of the average of the 3 groups of the two cases at normal and non-normal distributions for IgE, IgA and IgD

Table 3.14 represents the results of the average of IgE, IgA and IgD of the three groups of the two cases. The results show no significant differences ($P > 0.05$) in IgE, IgA and IgD of the three groups of the two cases.

TABLE 3.14 Comparison of the average of the 3 groups of the two cases at normal and non-normal distributions for IgE, IgA and IgD.

Parameter	Part A: Non final examinations period (n = 41)				
	Type of test	**Mean**	**SD**	**SE**	**P-value**
IgE (IU/mL)	Kruskal-Wallis	164.291	219.029	No result	0.346 NS
IgA (μg/dL)	Kruskal-Wallis	4.190	8.687	No result	0.471 NS
IgD (ng/mL)	Kruskal-Wallis	46.636	59.762	No result	0.482 NS
Parameter	**Part B: Final examinations period (n = 41)**				
	Type of test	**Mean**	**SD**	**SE**	**P-value**
IgE (IU/mL)	Kruskal-Wallis	180.563	212.429	No result	0.602 NS
IgA (μg/dL)	Kruskal-Wallis	2.882	10.041	No result	0.610 NS
IgD (ng/mL)	ANOVA	42.809	40.665	6.874	0.972 NS

NS = not significant at P-value ≥ 0.05

3.3 Section three: Categorized comparisons of the parameters (two categories)

The subjects were categorized using the Perceived Stress Questionnaire (PSQ) in each of the no examinations period and examinations period individually. Thus, the subjects were categorized twice (one at each period) producing the two categories for each of the cases. Categorization into the two groups was according to the total score of each subject in the questionnaire. Subjects with a total score below 75 (because 75 was resulted of the subtraction of the largest total score 120 from the smallest total score 30 and the result was divided by two then added to the smallest total score (120 – 30 = 90/2 = 45 + 30 = 75) were recognized as the low stress group and subjects with a total score equal to or more than 75 were recognized as the high stress group. Subsequently, the results of the two groups of the two cases were compared, with the no final examination period considered the control.

The examinations period had 41 subjects and this number of subjects was divided into two groups as follows: low stress group (n = 29, 70.7%) and high stress group (n = 12, 29.3%). The no examinations period also had 41 subjects divided into two groups as follows: low stress group (n = 32, 78%) and high or more than 75 were recognized as the high stress group. stress case (n = 9, 22%).

For the subjects during the no exam period, the categorization was carried on the subjects during that period and the results for the parameters were compared with the results for the same subjects during the exam period, not considering the categorization obtained for the exam period. This comparison is termed part A in all involved Tables. As for the subjects during the exam period,

as done above, the categorization was carried on the subjects during that period and the parameters were compared with the results for the same subjects in the no exam period, again not considering the categorization there. This comparison is termed part B in all involved Tables.

Paired samples t-test was used to determine the mean, standard deviation (SD), standard error (SE) and P-value for each parameter of each of the groups within the two cases.

3.3.1 The differential complete blood count

The counts of the total white blood cells, and their subtypes (neutrophils, eosinophils, basophils, lymphocytes and monocytes) were determined for the two groups of the two cases, as shown in Table 3.15.a and Table 3.15.b. Also, red blood cell and platelet counts along with the hemoglobin concentrations were determined for the two groups of the two cases, as shown in Table 3.15.c.

Using the paired samples t-test, the results show a highly significant increase ($P < 0.01$) in the white blood cells and neutrophils of the low and high stress groups of the two cases during no final examinations and during final examinations. There is a highly significant increase ($P < 0.01$) for lymphocytes of low stress group of the two cases during no final examinations and a significant increasing ($P < 0.05$) during final examinations and no significant changes ($P > 0.05$) for lymphocytes of the high stress group of the two cases during no final examinations and during final examinations but it tended towards a significant increase during final examinations ($P = 0.052$).

TABLE 3.15.a The differential complete blood count test results for the categorized (two groups) comparisons of the two cases using paired samples t-test.

Parameter	Group of stress level	Part A: Non final examination period (n = 41)		
		$Mean_1$ ± SD (± SE)	$Mean_2$ ± SD (± SE)	P-value
WBC (10^3/µL)	Low n = 32	6.63 ± 1.83 (± 0.32)	7.89 ± 1.82 (± 0.32)	0.000 HS
	High n = 9	6.42 ± 2.38 (± 0.79)	8.13 ± 1.81 (± 0.60)	0.002 HS
Neutrophils (10^3/µL)	Low n = 32	3.55 ± 1.33 (± 0.24)	4.44 ± 1.78 (± 0.31)	0.004 HS
	High n = 9	3.77 ± 1.65 (± 0.55)	5.16 ± 1.65 (± 0.55)	0.006 HS
Lymphocytes (10^3/µL)	Low n = 32	2.37 ± 0.72 (± 0.13)	2.74 ± 0.69 (± 0.12)	0.004 HS
	High n = 9	2.06 ± 0.81 (± 0.27)	2.32 ± 0.99 (±0.33)	0.162 NS
Parameter	**Group of stress level**	**Part B: Final examination period (n = 41)**		
		$Mean_1$ ± SD (± SE)	**$Mean_2$ ± SD (± SE)**	**P-value**
WBC (10^3/µL)	Low n = 29	6.51 ± 1.96 (± 0.37)	7.74 ± 1.69 (± 0.31)	0.001 HS
	High n = 12	6.77 ± 1.92 (± 0.56)	8.46 ± 2.03 (± 0.59)	0.003 HS
Neutrophils (10^3/µL)	Low n = 29	3.53 ± 1.46 (± 0.27)	4.44 ± 1.82 (± 0.34)	0.005 HS
	High n = 12	3.75 ± 1.25 (± 0.36)	4.98 ± 1.61 (± 0.47)	0.010 S
Lymphocytes (10^3/µL)	Low n = 29	2.26 ± 0.78 (± 0.14)	2.62 ± 0.78 (± 0.15)	0.010 S
	High n = 12	2.39 ± 0.67 (± 0.19)	2.72 ± 0.76 (± 0.22)	0.052 NS*

HS = high significant at P-value < 0.01
NS = not significant at P-value ≥ 0.05
* = tends to significance
$Mean_1$ = mean during non exams period
$Mean_2$ = mean during exams period

TABLE 3.15.b The differential complete blood count test results for the categorized (two groups) comparisons of the two cases using paired samples t-test.

Parameter	Group of stress level	Part A: Non final examination period (n = 41)		
		$Mean_1$ ± SD (± SE)	**$Mean_2$ ± SD (± SE)**	**P-value**
Monocytes (10^3/µL)	Low n = 32	0.50 ± 0.29 (± 0.05)	0.47 ± 0.24(± 0.04)	0.827 NS
	High n = 9	0.48 ± 0.21 (± 0.07)	0.51 ± 0.09 (± 0.03)	0.659 NS
Eosinophils (10^3/µL)	Low n = 32	0.17 ± 0.14 (± 0.03)	0.18 ± 0.13 (± 0.02)	0.876 NS
	High n = 9	0.12 ± 0.11 (± 0.04)	0.10 ± 0.09 (± 0.03)	0.347 NS
Basophils (10^3/µL)	Low n = 32	0.02 ± 0.04 (± 0.01)	0.03 ± 0.05 (± 0.01)	0.211 NS
	High n = 9	0.02 ± 0.04 (± 0.02)	0.03 ± 0.05 (± 0.02)	0.681 NS
Parameter	**Group of stress level**	**Part B: Final examination period (n = 41)**		
		$Mean_1$ ± SD (± SE)	**$Mean_2$ ± SD (± SE)**	**P-value**
Monocytes (10^3/µL)	Low n = 29	0.52 ± 0.30 (± 0.06)	0.48 ± 0.22 (± 0.04)	0.529 NS
	High n = 12	0.44 ± 0.17 (± 0.05)	0.53 ± 0.18 (± 0.05)	0.233 NS
Eosinophils (10^3/µL)	Low n = 29	0.17 ± 0.15 (± 0.03)	0.16 ± 0.12 (± 0.02)	0.712 NS
	High n = 12	0.15 ± .12 (± 0.03)	0.16 ± 0.16 (± 0.05)	0.809 NS
Basophils (10^3/µL)	Low n = 29	0.021 ± 0.041 (± 0.008)	0.024 ± 0.044 (± 0.008)	0.010 S
	High n = 12	0.02 ± 0.04 (± 0.01)	0.05 ± 0.05 (± 0.02)	0.052 NS*

S = significant at P-value < 0.05
NS = not significant at P-value ≥ 0.05
* = tends to significance
$Mean_1$ = mean during non exams period
$Mean_2$ = mean during exams period

TABLE 3.15.c The differential complete blood count test results for categorized (two groups) comparisons of the two cases using paired samples test.

Parameter	Group of stress level	Part A: Non final examination period (n = 41)		
		$Mean_1$ ± SD (± SE)	**$Mean_2$ ± SD (± SE)**	**P-value**
RBC (10^6/µL)	Low n = 32	4.420 ± 0.666 (±0.118)	4.64 ± 0.35 (± 0.06)	0.053 NS*
	High n = 9	4.470 ± 0.272 (±0.091)	4.58 ± 0.42 (± 0.14)	0.211 NS
HGB (g/dL)	Low n = 32	12.18 ± 2.156 (± 0.38)	12.49 ± 1.22 (± 0.22)	0.355 NS
	High n = 9	12.98 ± 0.69 (± 0.23)	12.82 ± 1.04 (± 0.35)	0.476 NS
PLT (10^3/µL)	Low n = 32	289.31 ± 83.15 (± 14.70)	298.44 ± 73.51 (± 12.99)	0.427 NS
	High n = 9	270.33 ± 54.16 (± 18.05)	282.89 ± 60.54 (± 20.18)	0.106 NS
Parameter	**Group of stress level**	**Part B: Final examination period (n = 41)**		
		$Mean_1$ ± SD (± SE)	**$Mean_2$ ± SD (± SE)**	**P-value**
RBC (10^6/µL)	Low n = 29	4.42 ± 0.71 (± 0.13)	4.64 ± 0.40 (± 0.07)	0.077 NS
	High n = 12	4.46 ± 0.15 (± 0.04)	4.59 ± 0.28 (± 0.08)	0.039 S
HGB (g/dL)	Low n = 29	12.15 ± 2.26 (± 0.42)	12.45 ± 1.24 (± 0.23)	0.421 NS
	High n = 12	12.85 ± 0.67 (± 0.19)	12.83 ± 0.99 (± 0.29)	0.893 NS
PLT (10^3/µL)	Low n = 29	284.62 ± 89.11 (±16.55)	295.69 ± 75.69 (± 14.06)	0.378 NS
	High n = 12	286.42 ± 40.37 (± 11.66)	293.42 ± 58.82 (± 16.98)	0.360 NS

S = significant at P-value < 0.05
NS = not significant at P-value ≥ 0.05
* = tends to significance
$Mean_1$ = mean during non exams period
$Mean_2$ = mean during exams period

Table 3.15.b shows no significant changes ($P > 0.05$) for monocytes and eosinophils of low and high stress groups of the two cases during no final examinations and during final examinations.

For basophils the results show no significant changes ($P > 0.05$) in basophils of the low and high stress level groups of the two cases during the no examinations period. As for the final examinations period, there is a significant increase ($P < 0.05$) in basophils of the low stress group of the two cases and no significant change ($P > 0.05$) in basophils of the low stress group of the two cases although it tended to a significant increase ($P = 0.052$).

Table 3.15.c shows no significant changes ($P > 0.05$) in red blood cells of the low stress group of the two cases during the no final examinations period and during the final examinations period, but it tends towards a significant increase ($P = 0.053$) during no examinations period. There is a significant increase ($P < 0.05$) in red blood cells of high stress group during final examinations period. Also, there are no significant changes ($P > 0.05$) in the hemoglobin concentrations and platelets counts of the low and high stress groups of the two cases during no final examinations period and final examinations period

3.3.2 Serum lipid profile

Table 3.16 shows the results of the serum levels of the lipid profile by using the paired samples t-test. The results demonstrate that there are no significant changes ($P > 0.05$) between the two cases of the two groups for the serum levels of cholesterol, triglycerides, low density lipoprotein (LDL), and low density lipoprotein (LDL), except for high stress group of the non final

TABLE 3.16 The serum levels of the lipid profile for the categorized (two groups) comparisons of the two cases using paired samples t-test.

Parameter (mmol/L)	Group of stress level	Part A: Non final examination period (n = 41)		
		$Mean_1$ ± SD (± SE)	$Mean_2$ ± SD (± SE)	P-value
Cholesterol	Low n = 32	4.22 ± 0.78 (± 0.12)	4.17 ± 0.65 (± 0.12)	0.411 NS
	High n = 9	4.27 ± 0.72 (± 0.24)	4.54 ± 0.85 (± 0.28)	0.197 NS
TG	Low n = 32	0.66 ± 0.23 (± 0.04)	0.65 ± 0.23 (± 0.04)	0.926 NS
	High n = 9	0.81 ± 0.39 (± 0.13)	0.99 ± 0.50 (± 0.17)	0.034 S
HDL	Low n = 32	1.86 ± 0.30 (± 0.05)	1.86 ± 0.29 (± 0.05)	0.893 NS
	High n = 9	2.00 ± 0.52(± 0.173)	1.93 ± 0.54 (± 0.18)	0.254 NS
LDL	Low n = 32	2.59 ± 0.73 (± 0.13)	2.65 ± 0.65 (± 0.12)	0.301 NS
	High n = 9	2.54 ± 0.58 (± 0.19)	2.83 ± 0.63 (± 0.21)	0.075 NS
Parameter	**Group of stress level**	**Part B: Final examination period (n = 41)**		
		$Mean_1$ ± SD (± SE)	**$Mean_2$ ± SD (± SE)**	**P-value**
Cholesterol	Low n = 29	4.14 ± 0.77 (± 0.14)	4.14 ± 0.66 (± 0.12)	0.945 NS
	High n = 12	4.46 ± 0.72 (± 0.21)	4.53 ± 0.75 (± 0.21)	0.717 NS
TG	Low n = 29	0.65 ± 0.27 (± 0.05)	0.65 ± 0.23 (± 0.04)	0.919 NS
	High n = 12	0.78 ± 0.27 (± 0.08)	0.92 ± 0.46 (± 0.13)	0.189 NS
HDL	Low n = 29	1.85 ± 0.30 (± 0.06)	1.82 ± 0.31 (± 0.06)	0.415 NS
	High n = 12	1.99 ± 0.46 (± 0.13)	1.99 ± 0.44 (± 0.13)	0.987 NS
LDL	Low n = 29	2.53 ± 0.68 (± 0.13)	2.62 ± 0.64 (± 0.12)	0.100 NS
	High n = 12	2.72 ± 0.73 (± 0.21)	2.87 ± 0.64 (± 0.19)	0.299 NS

NS = not significant at P-value ≥ 0.05 S= significant at P-value < 0.05
$Mean_1$= mean during non exams period $Mean_2$ = mean during exams period

examinations period which shows a highly significant increase (P = 0.034) in the triglycerides concentration.

3.3.3 The serum levels of cortisol and IL-6

Using the paired samples t-test, Table 3.17 demonstrates that there are highly significant increases (P < 0.01) in the cortisol concentrations of the low stress group of the two cases while there are no significant changes (P > 0.05) in cortisol concentrations of the high stress group of the two cases. Also, there were non significant changes (P > 0.05) in IL-6 concentration of the high and low stress groups of the two cases during final examinations case and no final examinations periods.

3.3.4 Serum levels of IgE, IgA, IgD, IgM and IgG antibodies

In Table 3.18 there are no significant differences (P > 0.05) observed in serum levels of IgE, IgA and IgD of the two groups of the two cases.

Since the results for the serum concentrations of the IgM and IgG antibodies are qualitative, Fisher's test was used for the analysis of the data. The results in Table 3.19 show that there is a significant increase (P < 0.05) for IgM concentration of the low stress level group of the no examinations period and no significant changes for IgM concentration of the low stress level group of the examinations period. A highly significant decrease (P < 0.01) is shown for IgG concentration of the low stress level groups of the two cases. There are no P-values for IgM and IgG concentrations of the high stress levels of the two cases because the percentages of significant of IgM and IgG concentrations were constant.

TABLE 3.17 The serum levels of cortisol and IL-6 for the categorized (two groups) comparisons of the two cases using paired samples t-test.

Parameter	Group of stress level	Part A: Non final examination period (n = 41)		
		$Mean_1$ ± SD (± SE)	$Mean_2$ ± SD (± SE)	P-value
Cortisol (µg/dL)	Low n = 32	9.26 ± 4.98 (± 0.88)	12.70 ± 3.90 (± 0.69)	0.006 [HS]
	High n = 9	5.99 ± 1.86 (± 0.62)	10.41 ± 6.56 (± 2.19)	0.108 [NS]
IL-6 (pg/mL)	Low n = 32	3.52 ± 8.42 (± 1.60)	5.72 ± 17.48 (± 3.30)	0.215 [NS]
	High n = 9	2.50 ± 1.75 (± 0.62)	2.79 ± 1.76 (± 0.62)	0.378 [NS]
Parameter	**Group of stress level**	**Part B: Final examination period (n = 41)**		
		$Mean_1$ ± SD (± SE)	**$Mean_2$ ± SD (± SE)**	**P-value**
Cortisol (µg/dL)	Low n = 29	8.84 ± 3.84 (± 0.71)	12.07 ± 4.02 (± 0.75)	0.005 [HS]
	High n = 12	7.83 ± 6.39 (± 1.85)	12.53 ± 6.02 (± 1.74)	0.092 [NS]
IL-6 (pg/mL)	Low n = 29	3.83 ± 8.92 (± 1.78)	5.90 ± 18.47 (± 3.69)	0.294 [NS]
	High n = 12	2.06 ± 0.83 (± 0.25)	3.17 ± 2.74 (± 0.83)	0.181 [NS]

HS = high significant at P-value < 0.01 NS = not significant at P-value ≥ 0.05
$Mean_1$= mean during non exams period $Mean_2$ = mean during exams period

TABLE 3.18 Serum levels of IgE, IgA and IgD for the categorized (two groups) comparisons of the two cases using paired samples t-test.

Parameter	Group of stress level	Part A: Non final examination period (n = 41)		
		$Mean_1$ ± SD (± SE)	$Mean_2$ ± SD (± SE)	P-value
IgE (IU/mL)	Low n = 32	197.78 ± 237.37 (± 41.96)	210.03 ± 230.76 (± 40.79)	0.960 NS
	High n = 9	45.23 ± 33.07 (± 11.02)	75.81 ± 58.87 (±19.62)	0.220 NS
IgA (µg/dL)	Low n = 32	3.18 ± 7.23 (±1.28)	3.40 ± 11.31 (± 2.00)	0.925 NS
	High n = 9	7.79± 12.49 (± 4.16)	1.03 ± 1.87 (± 0.62)	0.139 NS
IgD (ng/mL)	Low n = 32	51.21 ± 66.48 (± 13.30)	43.66 ± 40.92 (± 8.18)	0.282 NS
	High n = 9	35.39 ± 30.43 (± 11.50)	55.83 ± 41.94 (± 15.85)	0.193 NS
Parameter	**Group of stress level**	**Part B: Final examination period (n = 41)**		
		$Mean_1$ ± SD (± SE)	$Mean_2$ ± SD (± SE)	P-value
IgE (IU/mL)	Low n = 29	161.97 ± 238.14 (± 44.22)	180.73 ± 226.95 (± 42.14)	0.549 NS
	High n = 12	169.90 ± 173.33 (± 50.04)	180.15 ± 181.63 (± 52.43)	0.786 NS
IgA (µg/dL)	Low n = 29	3.49 ± 7.59 (± 1.41)	3.63 ± 11.87 (± 2.20)	0.956 NS
	High n =12	5.89 ± 11.10 (± 3.21)	1.07 ± 1.68 (± 0.49)	0.159 NS
IgD (ng/mL)	Low n = 29	54.33 ± 69.73 (± 14.54)	45.62 ± 43.45 (± 9.06)	0.240 NS
	High n = 12	30.93 ± 17.53 (± 5.84)	48.12 ± 35.32 (± 11.77)	0.184 NS

NS = not significant at P-value ≥ 0.05 $Mean_1$= mean during non exams period
$Mean_2$ = mean during exams period

TABLE 3.19 Serum levels of IgE, IgA and IgD for the categorized comparisons (two groups) of the two cases using Fisher's test.

Parameters (mg/dL)	Group of stress level	Part A: Non final examination period (n = 41)					Part B: Final examination period (n = 41)				
		No. of sig.	% No. of sig.	No. of non sig.	% No. of non sig.	P-value	No. of sig.	% No. of sig.	No. of non sig.	% No. of non sig.	P-value
IgM	Low	3	7.3%	29	70.7%	0.035 S	5	12.2%	24	58.5%	0.068 NS
	High	1	2.4%	8	19.5%	No result	0	0%	12	29.3%	No result
IgG	Low	6	14.6%	26	63.4%	0.002 HS	6	14.6%	23	56.1%	0.003 HS
	High	1	2.4%	8	19.5%	No result	1	2.4%	11	26.8%	No result

HS = high significant at P-value < 0.01 NS = not significant at P-value ≥ 0.05
S = Significant at P-value < 0.05

3.3.5 Comparison of the average of the two stress groups of the two cases at normal and non-normal distributions for the parameters

In this part the results of the average of the parameters for the low stress and high stress groups of the two cases (no final examinations case and final examinations case) are compared. Two statistical analyses were used: the ANOVA test for the parameters of normal distribution and the Kruskal-Wallis test for the parameters of non-normal distribution.

3.3.5.1 Comparison of the average of the two stress groups of the two cases at normal and non-normal distributions for CBC

Table 3.20 demonstrates the average of white blood cells, neutrophils, lymphocytes, monocytes, eosinophils, basophils, red blood cells, platelets counts, and hemoglobin concentrations for the low and high stress groups of the two cases. The results show no significant differences ($P > 0.05$) between them.

3.3.5.2 Comparison of the average of the two stress groups of the two cases at normal and non-normal distributions for lipid profile

The results (Table 3.21) of the average of cholesterol, high density lipoprotein (HDL) and high density lipoprotein (LDL) for the low and high stress groups of the two cases demonstrate no significant changes ($P > 0.05$) between them.

TABLE 3.20 Comparison of the average of the two stress groups of the two cases at normal and non-normal distributions for CBC.

Parameter	Part A: Non final examinations period (n = 41)				
	Type of test	Mean	SD	SE	P-value
WBC (10^3/µL)	ANOVA	6.585	1.932	0.302	0.778 NS
Neutrophils (10^3/µL)	ANOVA	3.595	1.387	0.217	0.680 NS
Lymphocytes (10^3/µL)	ANOVA	2.298	0.741	0.116	0.273 NS
Monocytes (10^3/µL)	Kruskal-Wallis	0.495	0.268	No result	0.713 NS
Eosinophils (10^3/µL)	Kruskal-Wallis	0.161	0.138	No result	0.341 NS
Basophils (10^3/µL)	Kruskal-Wallis	0.020	0.040	No result	0.819 NS
RBC (10^6/µL)	Kruskal-Wallis	4.431	0.599	No result	0.705 NS
HGB (g/dL)	Kruskal-Wallis	12.354	1.951	No result	0.549 NS
PLT (10^3/µL)	ANOVA	285.146	77.511	12.105	0.523 NS
Parameter	**Part B: Final examinations period (n = 41)**				
	Type of test	**Mean**	**SD**	**SE**	**P-value**
WBC (10^3/µL)	ANOVA	7.946	1.798	0.281	0.246 NS
Neutrophils (10^3/µL)	ANOVA	4.600	1.756	0.274	0.375 NS
Lymphocytes (10^3/µL)	ANOVA	2.646	0.767	0.120	0.711 NS
Monocytes (10^3/µL)	ANOVA	0.493	0.211	0.033	0.536 NS
Eosinophils (10^3/µL)	Kruskal-Wallis	0.159	0.128	No result	0.676 NS
Basophils (10^3/µL)	Kruskal-Wallis	0.032	0.047	No result	0.110 NS
RBC (10^6/µL)	ANOVA	4.625	0.364	0.057	0.719 NS
HGB (g/dL)	ANOVA	12.561	1.176	0.184	0.347 NS
PLT (10^3/µL)	ANOVA	295.024	70.448	11.002	0.927 NS

NS = not significant at P-value ≥ 0.05

TABLE 3.21 Comparison of the average of the two stress groups of the two cases at normal and non-normal distributions for lipid profile.

Parameter (mmol/L)	Part A: Non final examinations period (n = 41)				
	Type of test	**Mean**	**SD**	**SE**	**P-value**
Cholesterol	ANOVA	4.230	0.759	0.119	0.867 NS
TG	Kruskal-Wallis	0.691	0.272	No result	0.353 NS
HDL	ANOVA	1.891	0.357	0.056	0.292 NS
LDL	ANOVA	2.582	0.691	0.108	0.846 NS
Parameter (mmol/L)	**Part B: Final examinations period (n = 41)**				
	Type of test	**Mean**	**SD**	**SE**	**P-value**
Cholesterol	ANOVA	4.252	0.705	0.110	0.110 NS
TG	Kruskal-Wallis	0.726	0.331	No result	0.043 S
HDL	ANOVA	1.873	0.355	0.055	0.173 NS
LDL	ANOVA	2.691	0.642	0.100	0.257 NS

NS = not significant at P-value ≥ 0.05

The results of the average of triglycerides for the two groups of the final examinations case show significant changes ($P < 0.05$), but there are no significant changes ($P > 0.05$) in the average of triglycerides for the two groups of the final examinations case.

3.3.5.3 Comparison of the average of the two stress groups of the two cases at normal and non-normal distributions for cortisol and IL-6

Table 3.22 shows the average of cortisol hormone and IL-6 for the low and high stress groups of the two cases. The results show no significant changes ($P > 0.05$) in IL-6 for the two cases. The results of cortisol hormone show significant changes ($P < 0.05$) between the two groups of the no final examinations case but there are no significant changes ($P > 0.05$) in the average of cortisol between the two groups of the final examinations case.

3.3.5.4 Comparison of the average of the two stress groups of the two cases at normal and non-normal distributions for IgE, IgA and IgD

Table 3.23 demonstrates the average of IgE, IgA and IgD for the low and high stress groups of the two cases. The results show there are no significant changes ($P > 0.05$) for the three antibodies.

TABLE 3.22 Comparison of the average of the two stress groups of the two cases at normal and non-normal distributions for cortisol and IL-6.

Parameter	Part A: Non final examinations period (n = 41)				
	Type of test	Mean	SD	SE	P-value
Cortisol (µg/dl)	Kruskal-Wallis	8.542	4.664	No result	0.047 [S]
IL-6 (pg/mL)	Kruskal-Wallis	3.224	7.164	No result	0.290 [NS]
Parameter	**Part B: final examinations period (n = 41)**				
	Type of test	**Mean**	**SD**	**SE**	**P-value**
Cortisol (µg/dL)	ANOVA	12.200	4.618	0.721	0.776 [NS]
IL-6 (pg/mL)	Kruskal-Wallis	4.883	15.017	No result	0.340 [NS]

NS = not significant at P-value ≥ 0.05 S = significant at P-value < 0.05

TABLE 3.23 Comparison of the average of the two stress groups of two cases at normal and non-normal distributions for IgE, IgA and IgD.

Parameter	Part A: No final examinations period (n = 41)				
	Type of test	Mean	SD	SE	P-value
IgE (IU/mL)	Kruskal-Wallis	164.291	219.029	No result	0.083 NS
IgA (µg/dL)	Kruskal-Wallis	4.190	8.687	No result	0.078 NS
IgD (ng/mL)	Kruskal-Wallis	46.636	59.762	No result	0.725 NS
Parameter	Part B: Final examinations period (n = 41)				
	Type of test	Mean	SD	SE	P-value
IgE (IU/mL)	Kruskal-Wallis	180.563	212.429	No result	0.359 NS
IgA (µg/dL)	Kruskal-Wallis	2.882	10.041	No result	0.390 NS
IgD (ng/mL)	ANOVA	42.809	40.665	6.874	0.957 NS

NS = not significant at P-value ≥ 0.05

CHAPTER IV

Discussion

Chapter VI

Discussion

Under stressful conditions the hypothalamus releases releasing factor, which activates the anterior lobe of the pituitary gland to secrete adrenocorticotropin hormone in the blood circulation and when it reaches the adrenal gland, glucocorticoids, which modulate or regulate the immune response, are released (Shamsdin *et al*., 2010). Academic examinations represent one of the stressful events associated with a lowered immune system function and they have been used in stress research because they are predictable, standardized, and discrete examples of real-life stressors (Shamsdin *et al*., 2010). The research work presented here is aimed to study the effect of examinations stress on the immune system of female university students.

The parameters measured for the subjects of the study were assessed at a stressful time (examinations period) and a non-stressful time or condition (regular lectures, non-examinations period). The measured parameters were compared in three different ways. In the first section, parameters for the two cases (times or periods) were directly compared. In the second section, subjects for the two cases were classified into three categories (low, intermediate, and high stress level groups) according to the score obtained by each subject on the

Perceived Stress Questionnaire. Finally, in the third section, subjects for the two cases were classified into two categories (low and high stress groups) according to a cutoff score on the Perceived Stress Questionnaire.

In the first section of the results, highly significant increases are observed in the counts of total white blood cells (Non-examination period: 6.59 ± 1.93, Examination period: 7.95 ± 1.80), neutrophils (from 3.60 ± 1.39 to 4.60 ± 1.76), and lymphocytes (from 2.30 ± 0.74 to 2.65 ± 0.77) for the examination readings compared to the non-examination case (control). In the second section, highly significant increases for white blood cells counts are found for the two cases for the low stress level groups of the no examinations case (part A) (from 6.24 ± 1.86 to 7.53 ± 1.86) and of the examinations case (part B) (from 6.60 ± 1.54 to 7.50 ± 1.72), for the intermediate stress level groups of the no examinations case (part A) (from 7.22 ± 1.96 to 8.54 ± 1.69), and of the examinations case (part B) (from 6.64 ± 2.56 to 8.67 ± 1.88). A significant increase in the total white blood cells counts are observed for the high stress level group (from 6.42 ± 2.01 to 8.03 ± 1.62) of the examinations case (part B). There was no result for high stress level group of the no examinations (part A) case because the number of subjects was very small. For the neutrophile counts, significant increases were found for the two cases of the low level groups of the no examinations case (part A) (from 3.38 ± 1.35 to 4.29 ± 1.87) and of the examinations case (part B) (from 3.50 ± 1.33 to 4.21 ± 1.86), for the intermediate stress level groups of no examinations case (part A) (from 3.98 ± 1.44 to 5.04 ± 1.64), and of the examinations case (part B) (from 3.79 ± 1.54 to 5.15 ± 1.46). Also, significant increases were observed in lymphocytes counts of the no examinations case (part A) for the low stress level groups (from 2.24 ± 0.68 to 2.60 ± 0.57) and in the examinations case

(part B) for the intermediate stress level groups (from 2.25 ± 1.04 to 2.77 ± 1.07), monocyte counts (from 0.42 ± 0.24 to 0.56 ± 0.25) and basophile counts (from 0.01 ± 0.03 to 0.04 ± 0.05) during the examinations case (part B) for the intermediate stress level groups.

In the third section, there were highly significant increases for the low stress level groups of the two cases in the total white blood cells counts of the no examinations case (part A) (Non-examination period: 6.63 ± 1.83, Examination period: 7.89 ± 1.82) and of the examinations case (part B) (from 6.51 ± 1.96 to 7.74 ± 1.69), and neutrophile counts of the no examinations case (part A) (from 3.55 ± 1.33 to 4.44 ± 1.78) and of the examinations case (part B) (from 3.53 ± 1.46 to 4.44 ± 1.82). The high stress level group only showed increases in the total white blood cells counts of the no examinations case (part A) (from 6.42 ± 2.38 to 8.13 ± 1.81) and of the examinations case (part B) (from 6.77 ± 1.92 to 8.46 ± 2.03) and a significant increase in neutrophiles counts (from 3.75 ± 1.25 to 4.98 ± 1.61) for high stress level groups of the two cases during examinations case (part B). For lymphocyte counts, there was a highly significant increase in lymphocyte counts (from 2.37 ± 0.72 to 2.74 ± 0.69) of the two cases for the low stress level groups during the no examinations case (part A) and a significant increase in lymphocyte counts (from 2.26 ± 0.78 to 2.62 ± 0.78) of the two cases for the low stress level group during the examinations case (part B). Also, there was a significant increase in basophile counts (from 0.021 ± 0.041 to 0.024 ± 0.044) during the examinations case for the low stress level group.

This increase in white blood cells and its subtypes counts may be because during stress the brain sends signals to reverse the stress reaction leading to the secretion of stress hormones, which modulate the immunity by increasing

leukocyte trafficking and this is observed clearly in the results of final examinations case due to increased intensity of stress. This is similar to the study of Pruett (2003), which concluded that stress may lead to changes in the numbers and percentages of white blood cells and the stress of intense exercise induces increases in the blood concentrations of neutrophils, monocytes and lymphocytes. In another study (Bhatti and Shaikh, 2007), physiological stress (exercise stress) leads to significant increases in total white blood cell counts in both male and female students. On the other hand, the effects of stress which have been reported in studies on fish, amphibians, reptiles, birds, mice, rats, rabbits, foxes, horses, non-human primates, and humans showed a reduction in white blood cells numbers (Dhabhar, 2002). In contrast to animal studies, human studies have shown that stress can increase rather than decrease blood leukocyte numbers (Dhabhar, 2008).

In the present study, the results of the first section showed significant increases in the red blood cells counts (Non-examination period: 4.43 ± 0.60, Examination period: 4.63 ± 0.36) for the examinations case compared to the non-examinations case. In the second section, there were significant increases in the red blood cells counts (from 4.48 ± 0.16 to 4.71 ± 0.20) for the high stress groups during the examinations case (part B) and a highly significant increases in red blood cell counts (from 4.48 ± 0.27 to 4.64 ± 0.36) for the low stress groups during the examinations case (part B). In the third section, there was a significant increase in the red blood cell counts (from 4.46 ± 0.15 to 4.59 ± 0.28) for the high stress group during the examinations case (part B). Also, the results showed a highly significant decrease (from 12.55 ± 1.12 to 12.40 ± 1.25) in the hemoglobin concentration for the intermediate group during the no examinations

case (part A) of the second section while there were no significant changes in hemoglobin concentrations of the first and third sections.

This increase in red blood cells may be due to the increase of the breathing rate and heart beat rate during stress, which lead to the production of more oxygen-carrying red blood cells to get more oxygen. The decrease in the hemoglobin concentration for the intermediate group during no final examinations case may be a result of anemia because of the deficiency of iron which can be as a result of hereditary, loss of blood through heavy menstruation, or lack of iron in the diet. Researchers have reported that stress can cause a decrease of serum iron (Wei et *al*., 2008).

Wei et al in 2008 studied the characteristic effects of psychological stress on serum iron and erythropoiesis in rats and he found that hemoglobin was highly significantly decreased (consistent with this study) and red blood cells count was significantly decreased. Red blood cells numbers, hemoglobin, and hematocrit do not show decreases like white blood cells numbers (Dhabhar, 2002). Erythrocyte counts and hemoglobin concentrations remained unchanged during and after mental stress (Kondo and Morimoto, 1996). The effects of emotional stress on the hematological parameters were the increase in erythrocyte counts (consistent with this study) and hemoglobin concentrations (Jern *et al*., 1989). It was observed that there were only little changes in red blood cell counts as a result of psychological stress (Venkappa and Vasudeva, 2011). Thus, the variation of red blood cells production as a result of psychological stress in males and females needs to be further investigated (Venkappa and Vasudeva, 2011).

In the current study, cortisol concentration was highly significantly increased in the first section (Non-examination period: 8.54 ± 4.66, Examination period: 12.20 ± 4.62) for the examinations case compared to the control. In the second section, there were significant increases in the cortisol concentrations between the two cases during the no examinations case (part A) of the low stress level group (from 9.35 ± 5.40 to 13.16 ± 4.07) and of the intermediate stress level group (from 7.95 ± 3.60 to 11.72 ± 4.87) and there was a highly significant increase only in the low stress level group (from 8.91 ± 3.74 to 12.65 ± 3.10) between the two cases during the examinations case (part B). In the third section, there was a highly significant increase in the cortisol concentration only in the low stress level group between the two cases during the no examinations case (part A) (from 9.26 ± 4.98 to 12.70 ± 3.90) and during the examinations case (part B) (from 8.84 ± 3.84 to 12.07 ± 4.02).

The increase in the cortisol hormone due to stress is due to the fact that it is a stress hormone, which is elevated especially during stressful situations. This increase may be also because the cortisol level is at its highest level in the morning and at its lowest level a few hours after going to sleep and blood collection was done in the morning and that may explain the elevation of cortisol in the two cases. The study done by Shamsdin et al. (2010) to determine the effect of exam stress on serum cortisol level showed that exam stress resulted in a significant increase in the cortisol level. Another study done in 2006 by Weekes et al. on undergraduate students observed no significant correlations between elevations in psychological measures of stress and elevations in cortisol levels, thus no evidence was found to suggest a relationship between psychological and hormonal levels of stress. Therefore, examination stress can

increase stress hormones in stressful situations, but scores of anxiety standard questionnaire may be not useful for the determination of subject's anxious behavior (Sadeghi *et al.*, 2007).

In the current results, in the first section, there was a significant increase (P = 0.035) in IgM concentration and a highly significant decrease (P = 0.001) in IgG concentration for the examination case compared to the non-examinations case. In the second section, a significant increase (P = 0.022) was observed for IgM concentration of the intermediate stress level group during the no final examinations case (part A) and highly significant decreases were observed for IgG concentration of the intermediate stress level groups during the no final examinations case (part A) (P = 0.000) and during the final examinations case (part B) (P = 0.003). In the third section, there was a significant increase (P = 0.035) in IgM concentration of the low stress level group during the no examinations case (part A) and highly significant decreases in IgG concentration of the low stress level group during the no examinations case (part A) (P = 0.002) and during the examinations case (part B) (P = 0.003). There were no significant changes ($P \geq 0.05$) in IgE, IgA and IgD for the three sections.

The increase in IgM concentration only may be due to the fact that IgM antibodies are the major antibodies found in the primary immune response and thus they have an important function in the first line of defense against invading pathogens. Another factor may be that the subjects were all healthy and did not have any infections affecting the immune response.

A study done by Segal, et al. (2006) on twenty-five medical students concluded that there were no alterations in the IgA, IgM and IgG serum levels

during acute stress. Immunoglobulin levels were not significantly correlated with serum cortisol concentration (Vassend and Halvorsen, 1987). The production of IgG in mice was enhanced after acute stress and impaired in chronic stress (Silberman *et al*., 2003). The effect of exposure to emotional stress on the humoral immune function of rat induced the decrease in specific immunoglobulin G antibody level (Shao *et al*., 2003). The results of the effects of examination stress on serum concentrations of immunoglobulins on undergraduate students showed a small but significant decrease in IgM during the observed period and no other significant correlations between the psychological variables and IgA, IgG or IgE (Vassend and Halvorsen, 1987).

The present study showed no significant changes in IL-6 concentration ($P > 0.05$) for the three sections. Production of IL-6 and other proinflammatory cytokines can be directly stimulated by depression and other negative emotions and stressful experiences (Kiecolt-Glaser *et al*., 2003). Caregivers' average rate of increase in IL-6 was about four times as large as that of noncaregivers (Kiecolt-Glaser *et al*., 2003). Psychological stress predicted a greater expression of illness and an increased production of IL-6 in response to an upper respiratory infection (Cohen *et al*., 1999). Histochemical studies using rodent and human tissues have revealed that IL-6 and other cytokines are expressed in neuron cells within the central nerves system under non-inflammatory conditions in small quantities, but after infection or stress, they are expressed in much larger quantities and increased secretion of cytokines is involved in many pathological processes (Connor and Leonard, 1998). The study done by Starkweather (2007) to determine the influence of individuals' level of stress and mood on proinflammatory cytokines, reported significant improvements in stress, mood,

and quality of life in the people in the exercise group and they also reported a significant decrease in serum IL-6. Recent laboratory studies have also shown that acute psychological stress increases plasma interleukin-6 and fibrinogen concentrations in healthy individuals (Brydon and Steptoe, 2005).

This study showed no significant changes in lipid profile concentrations ($P > 0.05$) of the three sections, contradicting the results of most studies, which indicate that stress leads to increases in the lipid profile. Housewives are under more psychological and physiological stress as compared to lady health visitors and the levels of total cholesterol, low density lipoprotein (LDL) and triglycerides increased but high density lipoprotein (HDL) decreased with the increase in the level of stress (Wattoo *et al*., 2007). The study done by Bacon et al., (2004) on patients with suspected coronary artery disease provides evidence that exercise and mental stress lead to increases in lipids (total cholesterol, triglycerides, HDL and LDL) in patients with coronary artery disease. The exposure to short-term mental stress caused increases in the LDL, HDL and cholesterol concentrations in healthy young adults (Muldoon *et al*., 1995).

The findings in the present study show that the use of the total score on the Perceived Stress Questionnaire, in the second and third sections, was not useful and not appropriate for the determination of stress level. This may be due to the small size of the sample because it can be seen from the results that the highly significant changes appear usually in the low and intermediate groups respectively since they contain more numbers of subjects. In addition, another explanation may be that the questionnaire was not suitable for our society or it might not have been understood properly by the students. The questionnaire was

developed on a different society and, thus, for a different way of thinking and living.

CHAPTER V

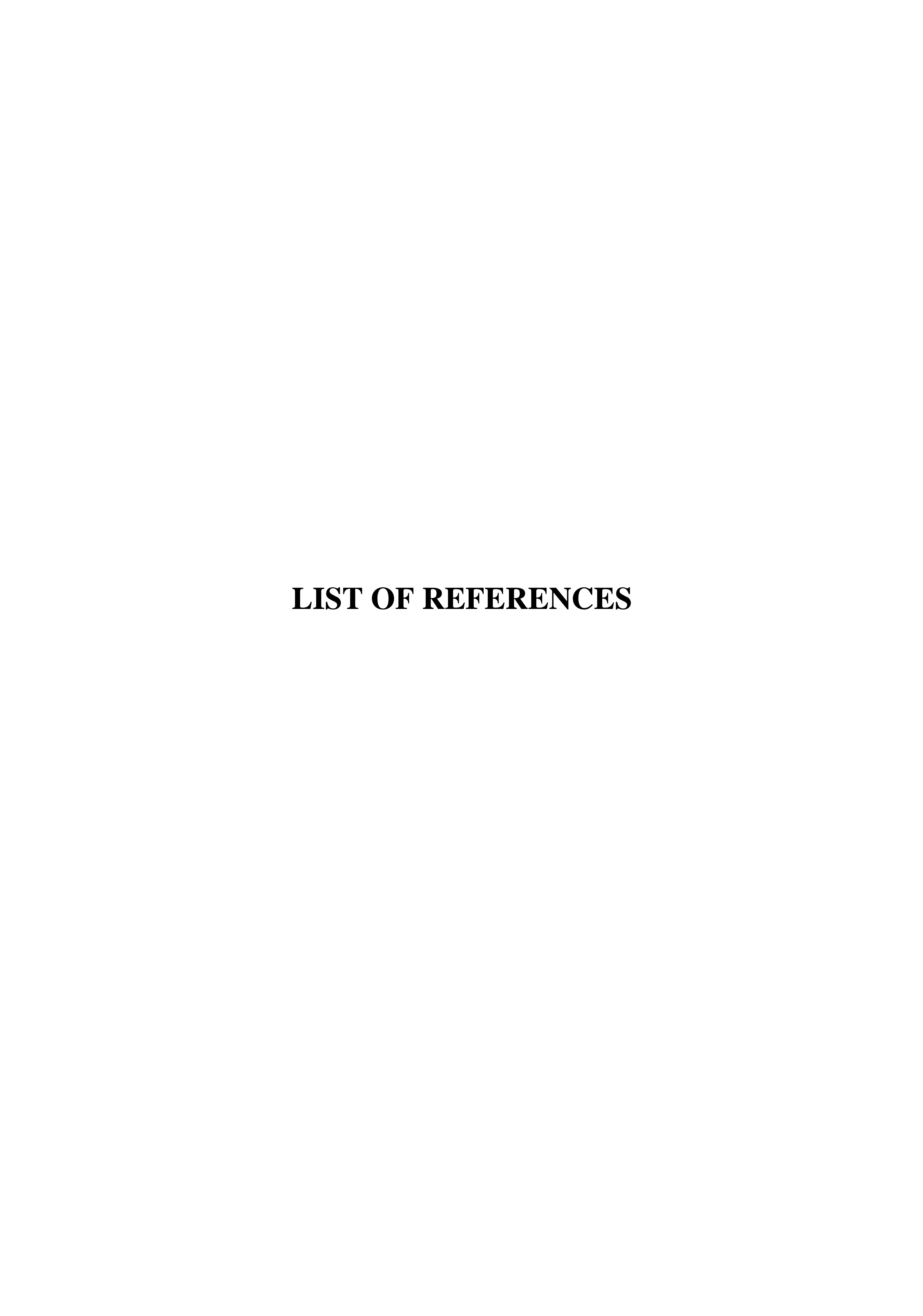
Conclusion and Recommendations

Chapter V

Conclusion and Recommendations

The findings of this study lead to the conclusion that acute psychological stress during examination period enhanced the immunity of the students.

It is recommended that further studies should be carried using a larger sample and different samples may be used, such as male university students, secondary school students, or one sample of male and female students to determine if there are gender differences in the response to stress. It is also recommended to construct a questionnaire that may be more appropriate for the local population.



LIST OF REFERENCES

LIST OF REFERENCES

Adekunle A. (2011) Dyslipidemic and Atherogenic Effects of Academic Stress. African Journal of Biochemistry Research, vol. 5: 356-359.

Agarwal, S. and Marshall, G. (2001) Stress Effects on Immunity and Its Application to Clinical Immunology. Clinical and Experimental Allergy, vol. 31: 25-31.

Ahmadi, S., Boroumand, M., Moghaddam, K., Tajik, P. and Dibaj, S. (2008) The Impact of Low Serum Triglyceride on LDL-Cholesterol Estimation. Archives of Iranian Medicine, vol. 11: 318-321.

Anupama, N., Sharma, M. Nagaraja, H. and Bhat, M. (2005) The Serum Immunoglobulin E Level Reflects The Severity of Bronchial Asthma. Thai Journal of Physiological Sciences, vol. 18: 35-40.

Arck, P., Rose, M., Hertwig, K., Hagen, E., Hildebrandt, M. and Klapp, B. (2001) Stress and Immune Mediators in Miscarriage. Human Reproduction, vol. 16: 1505-1511.

Arock, M., Schneider, E., Boissan, M., Tricottet., V. and Dy, M. (2002) Differentiation of Human Basophils: An Overview of Recent Advances and Pending Questions. Journal of Leukocyte Biology, vol. 71: 557-564.

Aronson, D. (2009) Cortisol - Its Role in Stress, Inflammation, and Indications for Diet Therapy. Today's Dietitian Journal, vol. 11: 38.

Atanackovic, D., Schnee, B., Schuch, G., Faltz, C., Schulze, J., Weber, C., Schafhausen, P., Bartels, K., Bokemeyer, C., Brunner-Weinzierl M. and Deter, H. (2006) Acute Psychological Stress Alerts the Adaptive Immune Response: Stress-Induced Mobilization of Effector T Cells. Journal of Neuroimmunology, vol. 176: 141-152.

Bacon, S., Ring, C., Lip, G. and Carroll, D. (2004) Increases in Lipids and Immune Cells in Response to Exercise and Mental Stress in Patients with Suspected Coronary Artery Disease: Effects of Adjustment for Shifts in Plasma Volume. Biological Psychology Journal, vol. 65: 237-250.

Bahrami, S. (2010) Influences of Culture and Social Class on Perception of Job Stress in Emerging Economies. International Review of Business Research Papers, vol. 6: 52-67.

Baig, A., Siddiqui, I., Naqvi, H., Sabir, S., Jabber, J. and Shahid, M. (2006) Correlation of Serum Cortisol Levels and Stress Among Medical Doctors Working in Emergency Departments. Journal of College of Physicians and Surgeons Pakistan, vol. 16: 576-580.

Bayazit, V. (2009) Evaluation of Cortisol and Stress in Captive Animals. Australian Journal of Basic and Applied Sciences, vol. 3: 1022-1031.

Behm, C. and Ovington, K. (2000) The Role of Eosinophils in Parasitic Helminth Infections: Insights from Genetically Modified Mice. Parasitology Today, vol. 16: 202-209.

Berger, S. and Toronto, M. (1970) Platelet Function: A Review. Part I. Normal Function. Canadian Medical Association Journal, vol. 102: 1271-1274.

Bevans, K., Cerbone, A. and Overstreet, S. (2008) Relations Between Recurrent Trauma Exposure and Recent Life Stress and Salivary Cortisol Among Children. Development and Psychopathology Journal, vol. 20: 257–272.

Bhatti, R. and Shaikh, D. (2007) The Effect of Exercise on Blood Parameters. Pakistan Journal of Physiology, vol. 3: 44-46.

Bijlani, R., Sud, S., Gandhi, B. and Tandon, B. (1986) Relationship of Examination Stress to Serum Lipid Profile. Indian Journal of Physiology and Pharmacology, vol. 30: 22-30.

Binder, C. (2010) Natural IgM Antibodies Against Oxidation-Specific Epitopes. Journal of Clinical Immunology, vol. 30: 56-60.

Blanco, J. and Garcia, M. (2008) Immune Response to Fungal Infections. Veterinary Immunology and Immunopathology, in press.

Boes, M. (2000) Role of Natural and Immune IgM Antibodies in Immune Responses. Molecular Immunology, vol. 37: 1141-1149.

Bremner, J., Vythilingam, M., Vermetten, E., Adil, J., Khan, S., Nazeer, A., Afzal, N., McGlashan, T., Elzinga, B., Anderson, G., Heninger, G., Southwick, S., and Charney, D. (2003) Cortisol Response to A Cognitive Stress Challenge in Posttraumatic Stress Disorder (PTSD) Related to Childhood Abuse. Psychoneuroendocrinology, vol. 28: 733-750.

Bremner, J. (2004) Brain? Understanding Trauma-Related Disorders from A Mind-Body Perspective. Directions in Psychiatry, vol. 24: 167-176.

Brigitta, B. (2002) Pathophysiology of Depression and Mechanisms of Treatment. Dialogues in Clinical Neuroscience, vol. 4: 7-20.

Brownlee, K., Moore, A. and Hackney, A. (2005) Relationship Between Circulating Cortisol and Testosterone: Influence of Physical Exercise. Journal of Sports Science and Medicine, vol. 4: 76-83.

Brydon, L. and Steptoe, A. (2005) Stress-Induced Increases in Interleukin-6 and Fibrinogen Predict Ambulatory Blood Pressure at 3-Year Follow-Up. Journal of Hypertension, vol. 23: 1001-1007.

Burke, H., Davis, M., Otte, C. and Mohr, D. (2005) Depression and Cortisol Responses to Psychological Stress: A Meta-Analysis. Psychoneuroendocrinology, vol. 30: 846-856.

Chandola, T., Britton, A., Brunner, E., Hemingway, H., Malik, M., Kumari, M., Badrick, E., Kivimaki, M. and Marmot, M. (2008) Work Stress and Coronary Heart Disease: What are The Mechanisms?. European Heart Journal, vol. 29: 640-648.

Chen, K. and Cerutti, A. (2011) The Function and Regulation of Immunoglobulin D. Current Opinion in Immunology, vol. 23: 1-8.

Chrousos, G. (2009) Stress and Disorders of The Stress System. Nature Reviews Endocrinology, vol. 5: 374-381.

Cohen, S., Doyle, W. and Skoner, D. (1999) Psychological Stress, Cytokine Production, and Severity of Upper Respiratory Illness. Psychosomatic Medicine, vol. 61: 175-180.

Cohen, S. Miller, G. and Rabin, B. (2001) Psychological Stress and Antibody Response to Immunization: A Critical Review of The Human Literature. Psychosomatic Medicine, vol. 63: 7-18.

Cohen, S., Janicki-Deverts, D. and Miller, G. (2007) Psychological Stress and Disease. The Journal of the American Medical Association, vol. 298: 1685-1687.

Colpo, A. (2005) LDL Cholesterol: "Bad" Cholesterol, or Bad Science?. Journal of American Physicians and Surgeons, vol. 10: 83-89.

Connor, T. and Leonard, B. (1998) Depression, Stress and Immunological Activation: The Role of Cytokines in Depressive Disorders. Life Sciences Journal, vol. 62: 583-606.

Connor, T. (2008) Don't Stress Out Your Immune System–Just Relax. Brain, Behavior, and Immunity, vol. 22: 1128-1129.

Cowen, P. (2002) Cortisol, Serotonin and Depression: All Stressed Out?. The British Journal of Psychiatry, vol. 180: 99-100.

Cruess, D., Antoni, M., McGregor, B., Kilbourn, K., Boyers, A., Alferi, S., Carver, C. and Kumar, M. (2002) Cognitive-Behavioral Stress Management Reduces Serum Cortisol by Enhancing Benefit Finding Among Women Being Treated for Early Stage Brest Cancer. Psychosomatic Medicine, vol. 62: 304-308.

Dhabhar, F. (2002) Stress-Induced Augmentation of Immune Function-The Role of Stress Hormones, Leukocyte Trafficking, and Cytokines. Brain, Behavior, and Immunity, vol. 16: 785-798.

Dhabhar, F. (2008) Enhancing Versus Suppressive Effects of Stress on Immune Function: Implications for Immunoprotection Versus Immunopathology. Allergy, Asthma and Clinical Immunology, vol. 4: 2-11.

Dhabhar, F., Saul, A., Daugherty, C., Holmes, T., Bouley, D. and Oberyszyn, T. (2010) Short-Term Stress Enhances Cellular Immunity and Increases Early Resistance to Squamous Cell Carcinoma. Brain, Behavior, and Immunity, vol. 24: 127-137.

Ehrenstein, M. and Notley, C. (2010) The Importance of Natural IgM: Scavenger, Protector and Regulator. Nature Reviews Immunology, vol. 10: 778-786.

Endresen, I., Vaernes, R., Ursin, H. and Tnder, O. (1987) Psychological Stress-Factors and Concentration of Immunoglobulins and Complement Components in Norwegian Nurses. Work and Stress: An International Journal of Work, Health and Organisations, vol. 1: 365-375.

Epel, E. (2009) Psychological and Metabolic Stress: A Recipe for Accelerated Cellular Aging?. Hormones Journal, vol. 8: 7-22.

Feghali, C. and Wright, T. (1997) Cytokines in Acute and Chronic Inflammation. Frontiers in Bioscience, vol. 2: 12-26.

Fliege, H., Rose, M., Arck, P., Walter, O., Kocalevent, R., Weber, C. and Klapp, B. (2005) The Perceived Stress Questionnaire (PSQ) Reconsidered: Validation and Reference Values From Different Clinical and Healthy Adult Samples. Psychosomatic Medicine, vol. 67: 78-88.

Gleich, G., Adolphson, C. and Leiferman, K. (1993) The Biology of The Eosinophilic Leukocyte. Annual Review of Immunology, vol. 44: 85-101.

Gordon, S. and Taylor, P. (2005) Monocyte and Macrophage Heterogeneity. Nature Reviews: Immunology, vol. 5: 953-964.

Gray, D., Gray, M. and Barr, T. (2007) Innate Responses of B cells. European Journal of Immunology, vol. 37: 3304-3310.

Guyton, A. and Hall, J. (2006) Textbook of Medical Physiology. 11th ed. Elsevier Saunders, Philadelphia, Pennsylvania, USA.

Haubenhofer, D. and Kirchengast, S. (2007) Dog Handlers' and Dogs' Emotional and Cortisol Secretion Responses Associated with Animal-Assisted Therapy Sessions. Society and Animals, 15: 127-150.

Hiremath, P. Bannigidad, P. and Geeta, S. (2010) Automated Identification and Classification of White Blood Cells (Leukocytes) in Digital Microscopic Images. International Journal of Computer Applications, vol. 6: 59-63.

Hussain D. (2010). Stress, Immunity, and Health: Research Findings and Implications. International Journal of Psychosocial Rehabilitation, vol. 15: 94-100.

Jemmott, J. and Magloire, K. (1988) Academic Stress, Social Support, and Secretory Immunoglobulin A. Journal of Personality and Social Psychology, vol. 55: 803-810.

Jern, C., Wadenvik, H., Mark, H., Hallgren, J. and Jern, S. (1989) Haematological Changes During Acute Mental Stress. British Journal of Haematology, vol. 71: 153-156.

Kai-Wen, C. (2009) A study of Stress Sources Among College Students in Taiwan. Journal of Academic and Business Ethics, vol. 2: 1-8.

Kalia, V., Sarkar, S., Gourley, T., Rouse, B. and Ahmed, R. (2006) Differentiation of Memory B and T cells. Current Opinion in Immunology, vol. 18: 255-264.

Kemeny, M. and Schedlowski, M. (2007) Understanding The Interaction Between Psychosocial Stress and Immune-Related Diseases: A Stepwise Progression. Brain, Behavior, and Immunity, vol. 21: 1009-1018.

Kiecolt-Glaser, J., Preacher, K., MacCallum, R., Atkinson, C., Malarkey, W. and Glaser, R. (2003) Chronic Stress and Age-Related Increases in The Proinflammatory Cytokine IL-6. Proceedings of the National Academy of Sciences, vol. 100: 9090-9095.

Kimura, K., Isowa, T., Ohira, H. and Murashima, S. (2005) Temporal Variation of Acute Stress Responses in Sympathetic Nervous and Immune Systems. Biological Psychology, vol. 70: 131-139.

King, S. and Hegadoren, K. (2002) Stress Hormones: How Do They Measure Up?. Biological Research For Nursing, vol. 4: 92-103.

Kishimoto, T. (2010) IL-6: From Its Discovery to Clinical Applications. International Immunology, vol. 22: 347-352.

Klinger, M. and Jelkmann, W. (2002) Review: Role of Blood Platelets in Infection and Inflammation. Journal of Interferon and Cytokine Research, vol. 22: 913-922.

Kobayashi, S. and DeLeo, F. (2009) Role of Neutrophils in Innate Immunity: A Systems Biology-Level Approach. Wiley Interdisciplinary Reviews: System Biology and Medicine, vol. 1: 309-333.

Kocalevent, R., Levenstein, S., Fliege, H., Schmid, G., Hinz, A., Brahler, E. and Klapp, B. (2007) Contribution to The Construct Validity of The Perceived Stress Questionnaire From A Population-Based Survey. Journal of Psychosomatic Research, vol. 63: 71-81.

Kompier, M., Cooper, C. and Geurts, S. (2000) A Multiple Case Study Approach to Work Stress Prevention in Europe. European Journal of Work and Organizational Psychology, vol. 9: 371-400.

Kondo, H. and Morimoto, K. (1996) Effects of Mental Arithmetic Stress on Blood Cell Counts and The Immune System. Environmental Health and Preventive Medicine, vol. 1: 76-79.

Kristensen, N., Madsen, A., Thomsen, A., and Christensen, J. (2004) Cytokine. Production By Virus-Specific CD8+ T Cells Varies with Activation State and Localization, But Not with TCR Avidity. Journal of General Virology, 85: 1703-1712.

Kunz-Ebrecht, S., Mohamed-Ali, V., Feldman, P., Kirschbaum, C. and Steptoe, A. (2003) Cortisol Responses to Mild Psychological Stress are Inversely Associated with Proinflammatory Cytokines. Brain, Behavior, and Immunity, vol. 17: 373-383.

Kyrou, I. and Tsigos, C. (2008) Chronic Stress, Visceral Obesity and Gonadal Dysfunction. Hormones, vol. 7: 287-293.

LeBien T. and Tedder, T. (2008) B lymphocytes: How They Develop and Function. Blood-Journal of The American Society of Hematology, vol. 112: 1570-1580.

Levenstein, S., Prantera, C., Varvo, V., Scribano, M., Berto, E., Luzi, C. and Andreoli, C. (1993) Development of The Perceived Stress Questionnaire: A New Tool for Psychosomatic Research. Journal of Psychosomatic Research, vol. 37: 19-32.

Lewis, B. (1959) The Metabolism of Cholesterol. Postgraduate Medical Journal, vol. 35: 208-215.

Lipman, N., Jackson, L., Trudel, L. and Weis-Garcia, F. (2005) Monoclonal Versus Polyclonal Antibodies: Distinguishing Characteristics, Applications, and Information Resources. Institute for Laboratory Animal Research Journal, vol. 46: 258-268.

Macpherson, A., McCoy, K., Johansen, F. and Brandtzaeg, P. (2008) The Immune Geography of IgA Induction and Function. Mucosal Immunology, vol. 1: 11-22.

Maes, M., Hendriks, D., Van Gastel, A., Demedts, P., Wauters, A., Neels, H., Janca, A. and Scharpe, S. (1997) Effects of Psychological Stress on Serum Immunoglobulin, Complement and Acute Phase Protein Concentrations in Normal Volunteers. Psychoneuoendocrinology, vol. 22: 397-409.

Maia, D., Marmar, C., Mendlowicz, M., Metzler, T., Nobrega, A., Peres, M., Coutinho, E., Volchan, E. and Figueira, I. (2008) Abnormal Serum Lipid Profile in Brazilian Police Officers with Post Traumatic Stress Disorder. Journal of Affective Disorders, vol. 107: 259-263.

Mantur, S. and Murthy, C. (2010) Effects of Academic Stress on Leukocyte Subset Distribution in Humans. Journal of Chinese Clinical Medicine, vol. 5: 516-519.

Marita, K. Kandregula, S. and Rao, L. (2012) Acute Noise Exposure Effect on Certain Hematological Parameters in Tailors: A Pilot Study. International Journal of Biological and Medical Research, vol. 3: 1529-1531.

Marsland, A., Bachen, E., Cohen, S., Rabin, B. and Manuck, S. (2002) Stress, Immune Reactivity and Susceptibility to Infectious Disease. Physiology and Behavior, vol. 77: 711-716.

McCann, B., Andrew, G., Benjamin, H., Wilkinson, C., Carter, J., Retzlaff, B., Russo, J. and Knopp, R. (1996) Variations in Plasma Lipid Concentration During Examination Stress. International Journal of Behavioral Medicine, vol. 3: 251-265.

McFarlane, E., Perez, C., Charmoy, M., Allenbach, C., Carter, K. Alexander, J. and Tacchini-Cottier, F. (2008) Neutrophils Contribute to Development of A Protective Immune Response During Onset of Infection with *Leishmania donovani*. Infection and Immunity, vol. 76: 532-541.

Meffre, E., Casellas, R. and Nussenzweig, M. (2000) Regulation of B Cell Development. Nature Immunology, vol. 1: 379-385.

Mendes, L., Piersma, T., Hasselquist, D., Matson, K.and Ricklefs, R. (2005) Variation in The Innate and Acquired Arms of The Immune System Among Five Shorebird Species. The Journal of Experimental Biology, vol. 209: 284-291.

Merz, C., Krantz, D. and Rozanski, A. (1993) Mental Stress and Myocardial Ischemia: Correlates and Potential Interventions. Texas Heart Institute Journal, vol. 20: 152-157.

Miller, N. (1990) HDL Metabolism and Its Role in Lipid Transport. European Heart Journal, vol. 11: 1-3.

Min, B., Brown, M. and LeGros, G. (2011) Understanding The Roles of Basophils: Breaking Dawn. Immunology Journal, vol. 135: 192-197.

Mitra, A. (2008) Diabetes and Stress: A Review. Ethnomedicine, vol. 2: 131-135.

Mojoyinola, J. (2008) Effects of Job Stress on Health, Personal and Work Behaviour of Nurses in Public Hospitals in Ibadan Metropolis, Nigeria. Ethnomedicine, vol. 2: 143-148.

Monteseirín, J. (2009) Neutrophils and Asthma. Journal of Investigational Allergology and Clinical Immunology, vol. 19: 340-354.

Morera, D. and MacKenzie S., (2011) Is There A Direct Role For Erythrocytes in The Immune Response?. Veterinary Research, vol. 42: 89-96.

Muldoon, M., Herbert, T., Patterson, S., Kameneva, M., Raible, R. and Manuck, S. (1995) Effects of Acute Psychological Stress on Serum Lipid Levels, Hemoconcentration, and Blood Viscosity. Archives of Internal Medicine, vol. 155: 615-620.

Murphy, L., Denis, R., Ward, C. and Tartar, J. (2010) Academic Stress Differentially Influences Perceived Stress, Salivary Cortisol, and Immunoglobulin-A in Undergraduate Students. Stress, vol. 13: 366-371.

Naik, S (2003) Introduction to The Immune System. Journal of Indian Rheumatology Association, vol. 11 : 8-13.

Nayanatara, A., Tripathi, Y., Nagaraja, H., Jeganathan, P., Ramaswamy, C., Ganaraja, B., Pai, S. and kamath, A. (2012) Effect of Chronic Immobilization Stress on Some Selected Physiological, Biochemical and Lipid Parameters in Wistar Albino Rats. Research Journal of Pharmaceutical, Biological and Chemical Sciences, vol. 3: 34-42.

O'Connor, A., Stephenson, T., Johnson, A., Wright, S., Tobin, M., Ratib, S. and Fielder, A. (2004) A Comparison of Findings on Parents' and Teachers' Questionnaires, and Detailed Ophthalmic and Psychological Assessments. Archives of Disease in Childhood, vol. 89: 831-835.

Ohl, L., Bernhardt, G., Pabst, O. and Förster, R. (2003) Chemokines As Organizers of Primary and Secondary Lymphoid Organs. Seminars In Immunology, vol. 15: 249-255.

Padgett, D. and Glaser, R. (2003) How Stress Influences The Immune Response. Trends in Immunology, vol. 24: 444-448.

Parkin, J. and Cohen B. (2001) An Overview of The Immune System. The Lancet, vol. 357: 1777-1789.

Parthasarathy, S., Steinberg, D. and Witztum, J. (1992) The Role of Oxidized Low-Density Lipoproteins in The Pathogenesis of Atherosclerosis. Annual Review of Medicine, vol. 43: 219-225.

Patterson, S., Gottdiener, J., Hecht, G., Vargot, S. and Krantz, D. (1993) Effects of Acute Mental Stress on Serum Lipids: Mediating Effects of Plasma Volume. Psychosomatic Medicine, vol. 55: 525-532.

Patterson, S., Matthews, K., Allen, M. and Owens, J. (1995) Stress-Induced Hemoconcentration of Blood Cells and Lipids in Healthy Women During Acute Psychological Stress. Health Psychology, vol. 14: 319-324.

Peters, M., Jacobs, S., Ehlers, M., Vollmer, P., Mfillberg, J., Wolf, E., Brem, G., Biischenfelde, K-H and Rose-John, S. (1996) The Function of The Soluble Interleukin 6 (IL-6) Receptor in Vivo: Sensitization of Human Soluble IL-6 Receptor Transgenic Mice Towards IL-6 and Prolongation of The Plasma Half- life of IL-6. The Journal of Experimental Medicine, vol. 183: 1399-1406.

Pfrieger, F. (2003) Cholesterol Homeostasis and Function in Neurons of The Central Nervous System. Cellular and Molecular Life Sciences, vol. 60: 1158-1171.

Pituch-Noworolska, A. and Błaut-Szlosarczyk, A. (2010) Immunoglobulins-Characteristics, Indications for Use and Clinical Effectiveness. International Review of Allergology and Clinical Immunology, vol. 16: 94-101.

Platts-Mills, T. (2001) The Role of Immunoglobulin E in Allergy and Asthma. American Journal of Respiratory and Critical Care Medicine, vol. 164: S1–S5.

Prelow, H. and Guarnaccia, C. (1997) Ethnic and Racial Differences in Life Stress Among High School Adolescents. Journal of Counseling and Development, vol. 75: 442-450.

Pruett, S. (2003) Stress and The Immune System. Pathophysiology, vol. 9: 133-153.

Putman, P. and Roelofs, K. (2011) Effects of Single Cortisol Administrations on Human Affect Reviewed: Coping with Stress Through Adaptive Regulation of Automatic Cognitive Processing. Psychoneuroendocrinology, vol. 36: 439-448.

Qureshi, G., Seehar, G., Zardari, M., Pirzado, Z. and Abbasi, S. (2009) Study of Blood Lipids, Cortisol and Haemodynamic Variations Under Stress in Male Adults. Journal of Ayub Medical College Abbottabad, vol. 21: 158-161.

Rader, D. (2006) Molecular Regulation of HDL Metabolism and Function: Implications for Novel Therapies. Journal of Clinical Investigation, vol. 116: 3090-3100.

Reaven, P. and Witztum, J. (1996) Oxidized Low Density Lipoproteins in Atherogenesis: Role of Dietary Modification. Annual Review of Nutrition, vol. 16: 51-71.

Rolfes, S., Pinna, K. and Whitney, E. (2006) Understanding Normal and Clinical Nutrition. 7th ed. Thomson/Wadsworth, Belmont, California, USA.

Rose-John, S., Dufhues, G., Graeve, L., Kruttgen, A., Lutticken, C., Mullberg, J., Pietzko, H., Schooltink, D., Stoyan, T., Dam, M., Zohlnhofer, D. and Heinrich, P. (1992) Studies on The Structure and Function of IL-6 and Its Receptor. Fresenius' Journal of Analytical Chemistry, vol. 343: 16.

Rosenberger, C., Elsenbruch, S., Scholle, A., De Greiff, A., Schedlowski, M., Forsting, M. and Gizewski, E. (2009) Effects of Psychological Stress on The Cerebral Processing of Visceral Stimuli in Healthy Women. Neurogastroenterol Motil, vol. 21: 740-745.

Rothenberg, M. and Hogan, S. (2006) The Eosinophil. Annual Review of Immunology, vol. 24: 147-174.

Sadeghi, Z., Eidi, M., Eidi, A. and Bahar, M. (2007) Effects of Practical Examination Stress on Serum ACTH and Cortisol Concentrations in Normal and Anxious Students. Journal of Medical Sciences, vol. 7: 489-491.

Samon, J. and Consuelo, A. (2006) The Relation of Ethnic Identity, Racial Identity, and Race-Related Stress Among African American College Students. Journal of College Students Development, vol. 47: 495-507.

Scanlan, J., Coe, C., Latts, A. and Suomi, S., (1987) Effects of Age, Rearing, and Separation Stress on Immunoglobulin Levels in Rhesus Monkeys. American Journal of Primatology, vol. 13: 11-22.

Schroeder, H. and Cavacini, L. (2010) Structure and Function of Immunoglobulins. The Journal of Allergy and Clinical Immunology, vol. 125: S41-S52.

Segal, A., Brunob, S. and Forte, W. (2006) Immune Function in Acute Stress. Allergol Immunopathol, vol. 34: 136-140.

Segerstrom, S. and Miller, G (2004) Psychological Stress and The Human Immune System: A Meta-Analytic Study of 30 Years of Inquiry. Psychological Bulletin, vol. 130: 601-630.

Selimuzzaman, S., Begum, N., Islam, N. and Begum, S. (2007) Effects of Surgical Stress on Serum Cortisol Level: A Comparative Study Between Elective and Emergency Surgery. Journal of Bangladesh Society of Physiologist, vol. 2: 28-33.

Sgoifo, A., Buwalda, B., Roos, M., Costoli, T., Merati, G. and Meerlo, P. (2006) Effects of Sleep Deprivation on Cardiac Autonomic and Pituitary-Adrenocortical Stress Reactivity in Rats. Psychoneuroendocrinology, vol. 31: 197-208.

Shamsdin, S. Anvar, M. and Mehrabani, D. (2010) The Effect of Exam Stress on Serum IL-6, Cortisol, CRP and IgE Levels. Iranian Red Crescent Medical Journal, vol. 12: 484-488.

Shao, F., Lin, W., Wang, W., Washington, W. and Zheng, L. (2003) The Effect of Emotional Stress on The Primary Humoral Immunity of Rats. Journal of Psychopharmacol, vol. 17: 179-183.

Sharpley, C. (2009) Neurobiological Pathways Between Chronic Stress and Depression: Dysregulated Adaptive Mechanisms?. Clinical Medicine: Psychiatry, vol. 2: 33-45.

Silberman, D., Wald, M. and Genaro, A. (2003) Acute and Chronic Stress Exert Effects on Antibody Responses Associated with Changes in Stress Opposing Hormone Regulation of T-Lymphocyte Reactivity. Journal of Neuroimmunology, vol. 144: 53-60.

Simpson, R., Hammacher, A., Smith, D., Matthews, J. and Ward, L. (1997) Interleukin-6: Structure-Function Relationships. Protein Science Journal, vol. 6: 929-955.

Slavin, L., Rainer, K., McCreary, M. and Gowda, K. (1991) Toward A Multicultural Model of The Stress Process. Journal of Counseling and Development, vol. 70: 156-163.

Snoeck, V., Peters, I. and Cox, E. (2006) The IgA System: A Comparison of Structure and Function in Different Species. Veterinary Research, vol. 37: 455-467.

Spencer, F. and Becker, R. (1997) Platelets: Structure, Function, and Their Fundamental Contribution to Hemostasis and Pathologic Thrombosis. Developments in Cardiovascular Medicine, vol. 193: 31-49.

Staines, N., Brostoff, J. and James, K. (1994) Introducing Immunology. 2nd ed., Mosby, Saint Louis, Missouri, USA.

Starkweather, A. (2007) The Effects of Exercise on Perceived Stress and IL-6 Levels Among Older Adults. Biological Research for Nursing, vol. 8: 186-194.

Steptoe, A., Willemsen, G., Owen, N., Flower, L. and Mohamed-Ali, V. (2001) Acute Mental Stress Elicits Delayed Increases in Circulating Inflammatory Cytokine Levels. Clinical Science, vol. 101: 185-192.

Steptoe, A., Hamer, M. and Chida, Y. (2007) The Effects of Acute Psychological Stress on Circulating Inflammatory Factors in Humans: A Review and Meta-Analysis. Brain, Behavior, and Immunity, vol. 21: 901-912.

Stowell, J. (2003) Use and Abuse of Academic Examinations in Stress Research. Psychosomatic Medicine, vol. 65: 1055-1057.

Sullivan, B. and Locksley, R. (2009) Basophils: A Nonredundant Contributor to Host Immunity. Journal of Immunity, vol. 1: 12-20.

Takase, B., Akima, T., Uehata, A., Ohsuzu, F. and Kurita, A. (2004) Effect of Chronic Stress and Sleep Deprivation on Both Flow-Mediated Dilation in The Brachial Artery and The Intracellular Magnesium Level in Humans. Clinical Cardiology, vol. 27: 223-227.

Taylor, C., Conrad, A., Wilhelm, F., Neri, E., DeLorenzo, A., Kramer, M., Giese-Davis, J., Roth, W., Oka, R., Cooke. J., Kramer, H. and Spiegel, D. (2006) Psychophysiological and Cortisol Responses to Psychological Stress in Depressed and Nondepressed Older Men and Women with Elevated Cardiovascular Disease Risk. Psychosomatic Medicine, vol. 68: 538-546.

Terwee, C., Bot, S., Boer, M., Windt, D., Knol, D., Dekker, J., Bouter, L. and Vet, H. (2007) Quality Criteria Were Proposed for Measurement Properties of Health Status Questionnaires. Journal of Clinical Epidemiology, vol. 60: 34-42.

Tirosh, A., Shai, I., Bitzur, R., Kochba, I., Tekes-Manova, D., Isaaeli, E., Shochat, T. and Rudich, A. (2008) Changes in Triglyceride Levels Over Time and Risk of Type 2 Diabetes in Young Men. Diabetes Care, vol. 31: 2032-2037.

Ursin, H., Mykletun, R., Tqnder, O., Vaernes, R., Relling, G., Isaksen, E. and Murison, R. (1984) Psychological Stress-Factors and Concentrations of Immunoglobulins and Complement Components in Humans. Scandinavian Journal of Psychology, vol. 25: 340-347.

Van Houdenhove, B. and Egle, U. (2004) Fibromyalgia: A Stress Disorder?. Psychotherapy Psychosomatics, vol. 73: 267-275.

Vassend, O. and Halvorsen R. (1987) Personality, Examination Stress and Serum Concentrations of Immunoglobulins. Scandinavian Journal of Psychology, vol. 28: 233-241.

Venkappa, S. and Vasudeva, C. (2011) Effect of Examination Stress on Red Blood Cells. Indian Journal of Public Health Research and Development, vol. 2: 122-124.

Vladutiu, A. (2000) Immunoglobulin D: Properties, Measurement, and Clinical Relevance. Clinical and Vaccine Immunology, vol. 7: 131-140.

Voehringer, D. (2009) The Role of Basophils in Helminth Infection. Trends in Parasitology, vol. 25: 551-556.

Wadsworth, E., Dhillon, K., Shaw, C., Bhui, K., Stansfeld, S. and Smith, A. (2007) Racial Discrimination, Ethnicity and Work Stress. Occupational Medicine, vol. 57: 18-24.

Wattoo, F., Memon, M., Memon, A., Wattoo, M., Tirmizi, S. and Iqbal, J. (2007) Effect of Stress on Serum Lipid Levels in Lady Health Visitors and Housewives. Rawal Medical Journal, vol. 32: 41-44.

Wattoo, F., Memon, M., Memon, A., Wattoo, M., Ghanghro, A., Yaquib, M. and Tirmizi, S. (2010) Effect of Stress on Serum Cholesterol Levels in Nurses and Housewives of Hyderabad-Pakistan. Pakistan Journal of Biochemistry and Molecular Biology, vol. 43: 50-53.

Weekes, N., Lewis, R., Patel, F., Garrison-Jakel, J., Berger, D. and Lupien, S. (2006) Examination Stress As An Ecological Inducer of Cortisol and Psychological Responses to Stress in Undergraduate Students. Stress, vol. 9: 199-206.

Wei, C., Zhou, J., Huang, X. and Li, M. (2008) Effects of Psychological Stress on Serum Iron and Erythropoiesis. International Journal of Hematology, vol. 88: 152-156.

Whitney E. and Rolfes S. (2005) Understanding Nutrition. 10th ed. Thomson/Wadsworth, Belmont, California, USA.

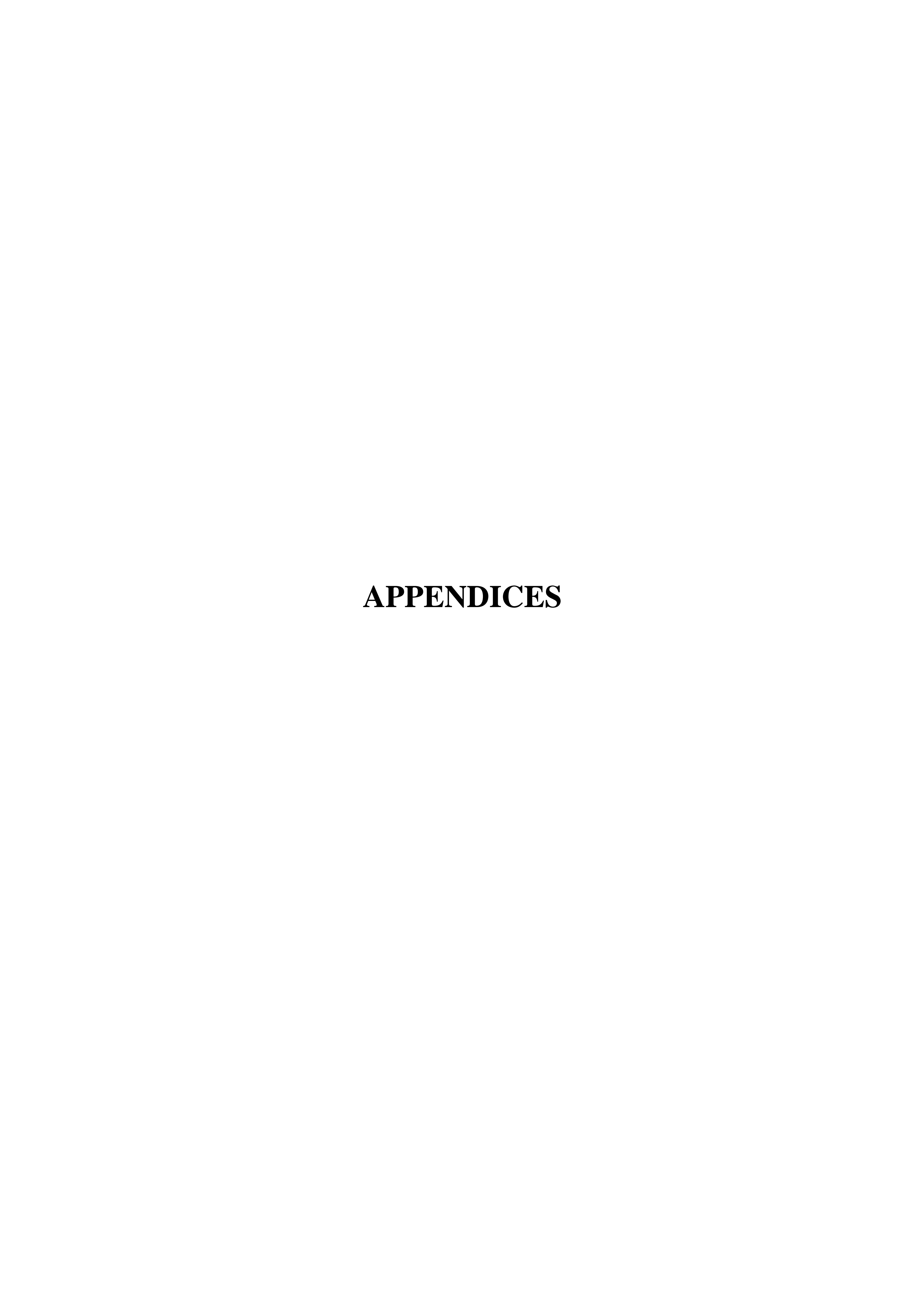
Woof, J. and Kerr ,M. (2006) The Function of Immunoglobulin A in Immunity. The Journal of Pathology, vol. 208: 270-282.

Woollard, K. and Geissmann, F. (2010) Monocytes in Atherosclerosis: Subsets and Functions. Nature Reviews Cardiology, vol. 7: 77-86.

Yang, E. and Glaser, R. (2002) Stress-Induced Immunomodulation and The Implications for Health. International Immunopharmacology, vol. 2: 315-324.

Yona, S. and Jung, S. (2010) Monocytes: Subsets, Origins, Fates and Functions. Current Opinion in Hematology, vol. 17: 53-59.

http://en.wikipedia.org/wiki/Haematopoiesis



APPENDICES

بســــــــــــم الله الرحمن الرحيم

هذا النموذج عبـــارة عن استبيان لدراسـة علمية لرسالة ماجستير تحت عنوان (الضغط النفسي والجهاز المناعي لدى طالبات الجامعة) فنرجو تعبئة هذا الاستبيان بكل دقة مع العلم أن هذه البيانات ستكون سرية و لن تستخدم إلا من أجل البحث العلمي و نشكر لكم حسن تعاونكم.

الاسم: الجنسية: العمر:

تاريخ الميلاد: التخصص: المستوى:

الحالة الإجتماعية: الوزن: الطول:

عدد الأولاد: محيط الخصر: محيط الورك:

رقم الجوال:

- هل تعاني من أي مرض من الأمراض التالية:

☐السكري ☐ أمراض مناعية ☐ ضغط دم

☐ أمراض وراثية ☐ فقر دم أو أمراض دم ☐ أخرى

حدديها...

- هل تمارسين الرياضة: ☐ نعم ☐ لا

إذا كانت الإجابة بنعم فما هي عدد الساعات التقريبية لممارسة الرياضة بالأسبوع.............

- هل تدخنين: ☐ نعم ☐ لا

حددي نوع التدخين:...

- هل تتعرضين للتدخين السلبي: ☐ نعم ☐ لا

عدد مرات تعرضك له باليوم..

- هل الدورة الشهرية منتظمة: ☐ نعم ☐ لا

إذا كانت الاجابه لا فما هو سبب عدم انتظامها...

- إذا كنت متزوجة هل أنت حامل: ☐ نعم ☐ لا

- هل تستخدمين موانع حمل: ☐ نعم ☐ لا

- هل تستخدمين أي من الأدوية التالية:

☐ منومات ☐ مهدئات ☐ للأعصاب ☐ للإكتئاب

☐ للحساسية ☐ للربو ☐ للضغط ☐ للسكري

- عدد ساعات النوم في الليل:

☐ 1-4 ساعات ☐ 4-8 ساعات ☐ 8 -10ساعات ☐ اكثر من 10 ساعات

- هل تعاني من اضطرابات النوم و الأرق: ☐ نعم ☐ لا

- هل تتعاطين أدويةهرمونية: ☐ نعم ☐ لا
إذا كنت تتعاطينها حدديها : ☐ مانع حمل ☐ أخرى..

- هل تتناولين المنبهات التالية: ☐ قهوة ☐ شاي ☐ مشروبات الطاقة

- حددي فترة تناولها: ☐ يوميا ☐ فترة الإمتحانات
حددي عدد المرات:...

- هل تتبعين حمية غذائية معينة: ☐ نعم ☐ لا
إذاكانت الإجابة نعم حدديها...

- ما هي أنواع الدهون والزيوت المستخدمة في الطعام رتبيها حسب كثرة الإستخدام:

☐ زيت الزيتون ☐ زيت الكانولا ☐ زيت الذرة ☐ زيت السمسم

☐ زيت دوار الشمس ☐ الزبدة ☐ السمنة

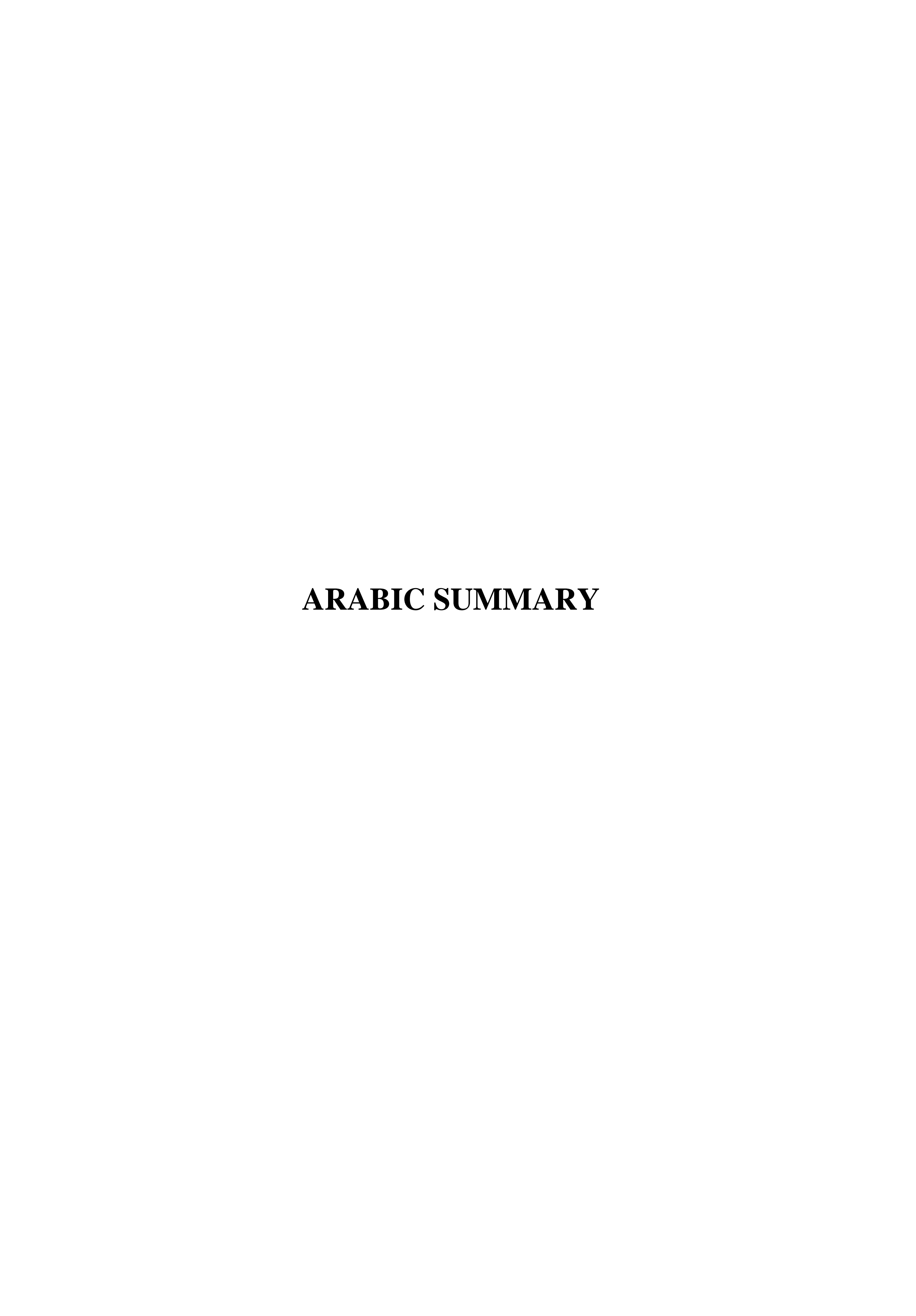
الترتيب من الأكثر للأقل:...

استبيان الإجهاد المحسوس

السؤال	تقريبا أبدا	أحيانا	غالبا	تقريبا دائما
1- أنت خائفة من المستقبلِ				
2- عِنْدَكَ العديد مِنْ الأمور المقلقة التي تشغل بالك				
3- مشاكلكَ تَبْدو كأنها تتراكم				
4- تَشعرين بالوحدة أو العُزْلة				
5- تَخَافُين بأنّك قدْ لا تَستطيعينُ إنْجاز أهدافِكَ				
6- تَجِدُين نفسك في حالاتِ نزاعِ أو عدم اتفاق				
7- أنت تحت ضغط من الناسِ الآخرين				
8- تَشعرين بالتحبيط				
9- تشعرين بأنك منتقدة أو محكومة				
10- تشعرين بالإحباط				
11- تَشْعرينُ بأنّك تَعْملينُ الأشياءَ لأنك يَجِبُ أنْ تعمليها لا لأنك تُريدينَ ذلك				
12- تَشْعرُين أنك محمّلة بالمسؤوليات				
13- لديكيَ الكثير مِنْ القراراتِ التي يجب تنفيذها				
14- تشعرين بالتعب				
15- تشعرين بالتوتر				
16- تَشْعرين بالراحة				
17- تشعرين بأنك مُنهَكة عقليا				
18- عِنْدَكَ مشكلةِ في الإسترخاء				
19- تشعرين بالهدوء				
20- أنت عصبية أو متذمّرة أو ضيقة الخلق				
21 تَشْعرين بأنّك تَعْملينُ أشياءَ تَحْبُّينها حقاً				
22- أنت تمتّعُين نفسك				
23- أنت مرحة				
24- أنت مليئة بالطاقةِ				
25- تَشْعرينُ بالأمان والحَماية				
26- لديكي عدد زائد مِنْ الأشياءِ لتَعمَليهاُ				
27- لديكي وقتُ كافيُ لنفسك				
28- تَشعرين بأنكُ تحت ضغط من المواعيد النهائية				
29- تَشْعرينُ بأنّك في عجلة من أمرك				
30- تَشْعرينُ بأن ّهناك الكثير مِنْ المطالب التي عليكي بذلها				

PERCEIVED STRESS QUESTIONNAIRE

Factor I: 41.6% explained variance (rotated solution)—scale "worries"
x You are afraid for the future
x You have many worries
x Your problems seem to be piling up
You feel lonely or isolated
x You fear you may not manage to attain your goals
You find yourself in situations of conflict
You are under pressure from other people
You feel discouraged
You feel criticized or judged
x You feel frustrated
You feel you're doing things because you have to not because you want to
You feel loaded down with responsibility
You have too many decisions to make
Factor II: 8.0% explained variance—scale "tension"
You feel tired
x You feel tense
x You feel rested
x You feel mentally exhausted
x You have trouble relaxing
x You feel calm
You are irritable or grouchy
Factor III: 5.0% explained variance—scale "joy"
x You feel you're doing things you really like
x You enjoy yourself
x You are lighthearted
x You are full of energy
x You feel safe and protected
Factor IV: 3.4% explained variance—scale "demands"
x You have too many things to do
x You have enough time for yourself
x You feel under pressure from deadlines
x You feel you're in a hurry
x You feel that too many demands are being made on you



ARABIC SUMMARY

الملخص العربي

الضغط النفسي هو عبارة عن الشعور بفقد التوازن الذي يتعرض له الفرد عند تعرضه لأحداث معينة مما قد يحد من قدرته على القيام بمهامه كما ينبغي و يقلل من قدرته على التركيز وعلى النوم والاسترخاء و يؤدي إلى سرعة شعوره بالتعب والإجهاد والتوتر والغضب. وهذه الأحداث ممكن أن تكون ناتجة عن الإصابة بمرض معين أو وفاة وفقدان شخص عزيز أو قد تنجم عن ظروف العمل وضغوطاته أو مشاكل زوجية أو أسرية أو اجتماعية و هناك أيضا أسباب أخرى قد تؤدي إلى الضغط النفسي كسوء التغذية و الإفراط في تناول العقاقير و المضادات الحيوية.

الضغط النفسي إما أن يكون حاد أو مزمن، فالضغط النفسي الحاد هو الذي يستمر لفترة قصيرة أي ساعات أو أيام، أما الضغط النفسي المزمن هو الذي يستمر لفترة طويلة أي أسابيع أو شهور. عدة دراسات تشير إلى أن الضغط النفسي يحفز الجسم لإفراز هرمون الكورتيزول من قشرة الغدة الكظرية في محاولة منه لمواجهة الضغوط واستعادة توازن الجسم واستمرار إفراز هذا الهرمون يؤدي إلى إضعاف المناعة و يؤدي للإصابة بالأمراض العضوية كالسكري و مرض ضغط الدم وأمراض القلب والسرطان و السمنة و أيضا يؤدي إلى الإصابة بأمراض نفسية كالقلق والاكتئاب والحرمان من النوم والأرق.

هناك دراسات أخرى تشير إلى أن الضغط النفسي الحاد يؤدي إلى تحفيز الجهاز المناعي حيث أن إفراز هرمون الكورتيزول لفترة قصيرة يؤدي إلى تنشيط و تحفيز الخلايا المناعية في الجسم لتقوم بوظيفتها المناعية بينما في حالة الضغط النفسي المزمن فإن استمرار إفراز هذا الهرمون لفترة طويلة يؤدي إلى عدم استجابة الخلايا المناعية له وبالتالي يؤدي إلى تثبيط هذه الخلايا وإضعافها مما ينتج عنه حدوث الأمراض.

تتناول هذه الدراسة تأثير الضغط النفسي الحاد الذي يتمثل بالامتحانات النهائية على الجهاز المناعي. فكما هو معلوم أن الطلاب والطالبات يكونون أكثر عرضة للضغوط النفسية خصوصا في نهاية العام الدراسي وأثناء فترة الامتحانات النهائية. وقد أجريت

الدراسة على إحدى و أربعين طالبة من طالبات جامعة الملك عبد العزيز حالتهن الصحية جيدة وأعمارهن تتراوح بين التاسعة عشر و السادسة والعشرون.

تم أخذ عينات الدم من الطالبات مرتين، المرة الأولى في بداية العام الدراسي حيث تكون خالية من ضغوط الامتحانات، والمرة الثانية في نهاية العام الدراسي أثناء الامتحانات النهائية حيث تكون هذه الفترة مليئة بالضغوط. وقد أجريت التحاليل التالية على العينات في المرتين: قياس عدد خلايا الدم البيضاء بأنواعها (أحادية النوى، واللمفاوية، والقاعدية الاصطباغ، والحامضية الاصطباغ، والمتعادلة الاصطباغ) وقياس عدد خلايا الدم الحمراء وقياس تركيز كل من الهيموجلوبين والانترليوكين-6 و هرمون الكورتيزول وتركيز الدهون (الكولسترول، والدهون ثلاثية الجلسرول، والليبوبروتينات منخفضة الكثافة، والليبوبروتينات عالية الكثافة) وقياس تركيز الأجسام المضادة (IgM، IgG، IgA، IgD، و IgE) و تمت مقارنة نتائج الفترتين في القسم الأول للنتائج.

في القسم الثاني للنتائج تم تقسيم الطالبات في كلتا الفترتين إلى ثلاث مجموعات: مجموعة مستوى الضغط النفسي المنخفض، مجموعة مستوى الضغط النفسي المتوسط و مجموعة مستوى الضغط النفسي العالي وفقا لاستبيان الضغط المحسوس وتمت مقارنة نتائج كل مستوى للفترتين. في القسم الثالث للنتائج تم تقسيم الطالبات في كلتا الفترتين إلى مجموعتين مجموعة مستوى الضغط النفسي المنخفض و مجموعة مستوى الضغط النفسي العالي وفقا لاستبيان الضغط المحسوس وتمت مقارنة نتائج كل مستوى للفترتين.

ومن أهم النتائج المستخلصة هو أن هناك زيادة معنوية كبيرة في عدد خلايا الدم البيضاء، وعدد الخلايا متعادلة الاصطباغ، و عدد الخلايا اللمفاوية وتركيز هرمون الكورتيزول كما وجد أن هناك زيادة معنوية في عدد خلايا الدم الحمراء و تركيز الأجسام المضادة إم بينما هناك إنخفاض معنوي كبير في تركيز الأجسام المضادة جي في القسم الأول.

في القسم الثاني، لوحظ أن هناك زيادة معنوية كبيرة في عدد خلايا الدم البيضاء لمجموعات مستوى الضغط المنخفض والمتوسط لكلا الحالتين و زيادة معنوية لمجموعة مستوى الضغط النفسي العالي أثناء فترة الامتحانات النهائية. هناك زيادة معنوية في عدد الخلايا متعادلة الاصطباغ البيضاء لمجموعتي مستوى الضغط النفسي المنخفض والمتوسط لكلا الحالتين وفي عدد الخلايا اللمفاوية فقط لمجموعتي مستوى الضغط النفسي العالي لكلتا

الحالتين. كما وجد أن هناك زيادة معنوية في عدد الخلايا أحادية النوى والخلايا قاعدية الاصطباغ فقط لمجموعة مستوى الضغط النفسي المتوسط أثناء فترة الامتحانات النهائية. هناك زيادة معنوية كبيرة في عدد خلايا الدم الحمراء لمجموعة مستوى الضغط النفسي المنخفض و زيادة معنوية لمجموعة الضغط النفسي العالي أثناء فترة الامتحانات النهائية. لوحظ نقص معنوي كبير في تركيز الهيموجلوبين لمجموعة مستوى الضغط النفسي المتوسط أثناء فترة عدم وجود الامتحانات النهائية. هناك زيادة معنوية في هرمون الكورتيزول لمجموعتي مستوى الضغط النفسي المنخفض و المتوسط أثناء فترة عدم وجود الامتحانات النهائية و زيادة معنوية كبيرة لمجموعة مستوى الضغط النفسي المنخفض أثناء فترة الامتحانات النهائية.ووجد أن هناك زيادة معنوية في تركيز الأجسام المضادة إم فقط لمجموعة مستوى الضغط النفسي المتوسط أثناء فترة عدم وجود الامتحانات النهائية و نقص معنوي كبير معنوية في تركيز الأجسام المضادة جي فقط لمجموعتي مستوى الضغط النفسي المتوسط لكلتا الحالتين.

في القسم الثالث، وجد أن هناك زيادة معنوية كبيرة في عدد خلايا الدم البيضاء لمجموعات مستوى الضغط النفسي المنخفض والعالي لكلتا الحالتين و زيادة معنوية كبيرة في عدد الخلايا المتعادلة لمجموعتي مستوى الضغط النفسي المنخفض في كلتا الحالتين و لمجموعة مستوى الضغط النفسي العالي أثناء فترة عدم وجود الامتحانات النهائية، بينما هناك زيادة معنوية لمجموعة مستوى الضغط النفسي العالي أثناء فترة الامتحانات النهائية. وقد لوحظ أن هناك زيادة معنوية كبيرة في عدد الخلايا اللمفاوية لمجموعة مستوى الضغط النفسي العالي أثناء فترة عدم وجود الامتحانات النهائية، بينما هناك زيادة معنوية لمجموعة مستوى الضغط النفسي المنخفض أثناء فترة الامتحانات النهائية. ووجدت هناك زيادة معنوية في عدد الخلايا قاعدية الاصطباغ فقط لمجموعة مستوى الضغط النفسي المنخفض أثناء فترة الامتحانات النهائية وهناك زيادة معنوية في خلايا الدم الحمراء فقط لمجموعة مستوى الضغط النفسي العالي أثناء فترة الامتحانات النهائية. كما لوحظت زيادة معنوية كبيرة في تركيز هرمون الكورتيزول لمجموعتي مستوى الضغط النفسي المنخفض في كلا الحالتين و زيادة معنوية في تركيز الأجسام المضادة إم لمجموعة مستوى الضغط النفسي المنخفض أثناء فترة عدم وجود الامتحانات النهائية و نقص معنوي كبير في تركيز الأجسام المضادة جي لمجموعة مستوى الضغط النفسي المنخفض في كلا الحالتين. كما أنه لم يلاحظ أن هناك فروق معنوية في تركيز الإنترليوكين-6 و تركيز الدهون (الكولسترول، والدهون

ثلاثية الجلسرول، والليبوبروتينات منخفضة الكثافة، والليبوبروتينات عالية الكثافة). وقد خلصت هذه الدراسة و خصوصا بالاعتماد على القسم الأول بدون استخدام الاستبيان أن الضغط النفسي الحاد أثناء فترة الامتحانات عزز المناعة لدى الطالبات.

يوصى بالقيام بالمزيد من الدراسات في هذا الموضوع باستخدام استبيانات موصى بها من قبل اختصاصيين من نفس المجتمع والبيئة. كما يجب أن يكون حجم العينة أكبر و يمكن تطبيق الدراسة على عينات أخرى مثل طلاب الجامعة الذكور، طلاب المدارس الثانوية ذكورا أو إناثا أو عينة واحدة من الطلاب والطالبات لإظهار الفروق بينهما و ما إذا كان هناك علاقة بين الضغط النفسي والاختلافات بين الجنسين.

الملخص العربي

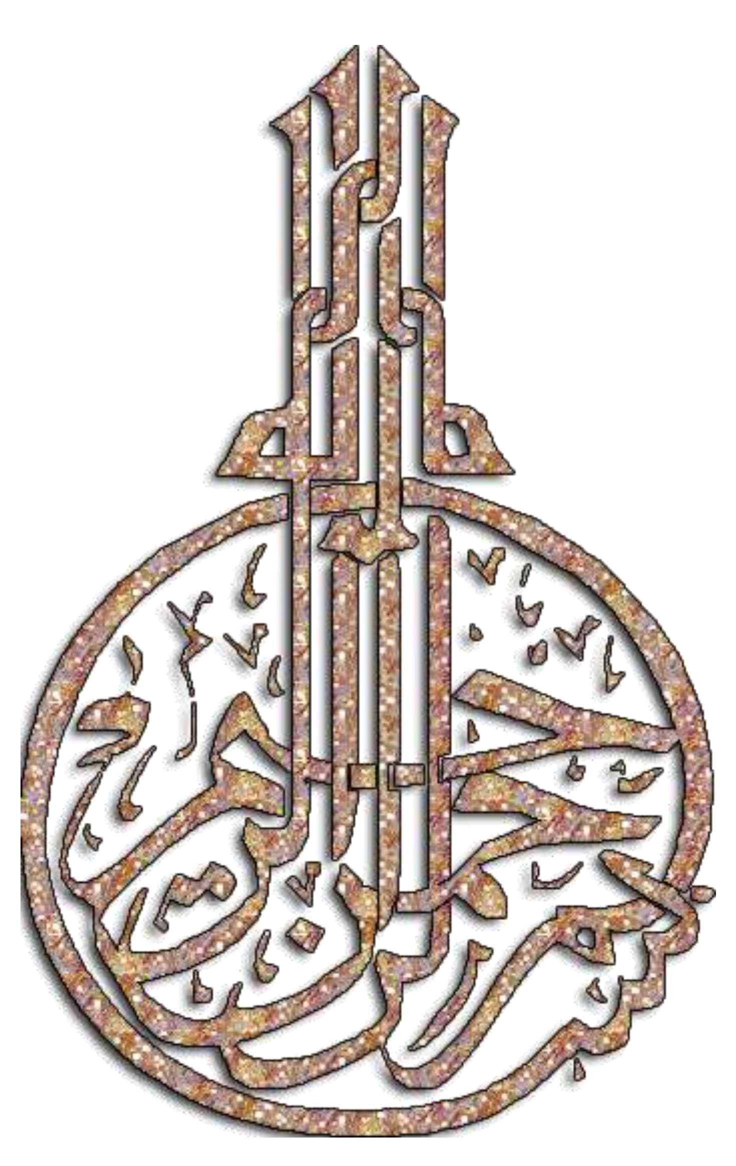
الضغط النفسي والجهاز المناعي لدى طالبات الجامعة

إعداد
أفراح عبدالله إسكندر

بحث مقدم لنيل درجة الماجستير في العلوم
(الكيمياء الحيوية)

باشراف
د. سوسن حسان محاسني

كلية العلوم
جامعة الملك عبد العزيز
جدة-المملكة العربية السعودية
جمادى الأول ١٤٣٤هـ - أبريل ٢٠١٣م


بسم الله الرحمن الرحيم

الضغط النفسي والجهاز المناعي لدى طالبات الجامعة

إعداد

أفراح عبدالله إسكندر

بحث مقدم لنيل درجة الماجستير في العلوم

(الكيمياء الحيوية)

بإشراف

د. سوسن حسان محاسني

كلية العلوم

جامعة الملك عبد العزيز

جدة-المملكة العربية السعودية

جمادى الأول ١٤٣٤هـ - أبريل ٢٠١٣م