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and Scientific Research
University of Al-Qadisiyah
College of Medicine**



Human Leukocytes Antigen Class I and Cytokines Profile in Schizophrenic Patients

A Thesis

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Philosophy Doctorate in Medical Microbiology**

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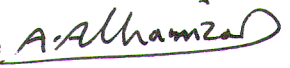
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Dedication

To my family , soul of my father and
my dear old brother, my mother ,
brothers and sisters.

Israa

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Summary:

Schizophrenia is a severe mental disorder that affects approximately one percent of the general population. It is a complex, chronic mental health disorder characterized by a spectrum of symptoms, including delusions, hallucinations, disorganized speech or behavior, and impaired cognitive ability.

This case-control study was arranged to investigate the possible role of selected genetic, inflammatory and endocrine parameters in a random sample of patient with schizophrenia in the Al Diwanyia province. Ten ml blood samples obtained from Sixty patients with chronic schizophrenia attending the outpatient department of psychiatry in Al Diwaniyia teaching hospital have been recruited in the study and compared to 30 first degree relative subjects and to 30 unrelated health control, there was 46 male and 14 female with male to female ratio 3.28 :1, their ages ranged between 14-75 years, 6ml blood samples were assessed for serum measurement of cortisol , adrenocorticotrophic hormone using Tosoh AIA-360 immunoassay analyzer, and for measurement of serum human Corticotropin Releasing Hormone , neuregulin 1 and interleukins 6&10 were accomplished by Enzyme-Linked Immunosorbent Assay technique. Two ml blood sample was utilized for genomic DNA extraction from peripheral blood leukocytes for detection of single nucleotide polymorphism of *Methylene tetra - hydrofolate Reductase* and *D-Amino Acid Oxidase Activator* gene using polymerase chain reaction-restriction fragment length polymorphism technique. Another 2 ml blood sample was also subjected for genomic DNA extraction for human leukocyte antigen typing using sequence-specific primer technologies.

Summary

Results showed no statistically significant differences in mean age ,between the 3 study groups. Males were more frequent but not statistically significant than females. Half of patients with schizophrenia were in the early adulthood at the time of diagnosis and 70% of patients gave a positive family history .

The mean serum cortisol , adrenocorticotrophic and neuregulin hormone with interleukins 6 were significantly higher in patients as compared to both control groups P value <0.001. While serum human corticotropin releasing hormone and interleukins 10 on the other hand showed only a marginal and statistically insignificant increase in schizophrenic patients when compared to both control groups.

Human leukocyte antigen A&B typing were used to predict the risk of having schizophrenia in which one human leukocyte antigen A gene (A*03) and two human leukocyte antigen B genes (B*07 and B*40) are significantly increased the risk of having schizophrenia compared to general population controls P value = 0.005, 0.005and 0.027 respectively. Another four human leukocyte antigen A genes, A*23, A*26, A*31 and A*68 and one human leukocyte antigenB gene (B*44) had a protective effect and significantly decreased the risk of having schizophrenia by 7.3, 14.5, 3.2, 8.8 and 20.5 times respectively. Human leukocyte antigen - B*40 gene increased the risk of having the disease by 3.3 times, while B42 decreased the risk by 4 times however fail to reach the statistical significance.

Among the studied two candidate susceptibility genes, *Methylenetetrahydrofolate Reductase* genotypes had significant predictive power. The T allele had the strongest association P<0.001and significantly increases the risk of having schizophrenia compared to general population control. While the C allele had a significant protective

Summary

effect. Both the heterozygous CT and the homozygous TT genotypes increase the risk of the disease by 7.1, $P < 0.001$ and 5.8, $P < 0.001$ times respectively. While the wild CC gene showed a statistically significant protective effect. Its occurrence reduces the risk of having schizophrenia by 16 times. No important or statistically significant association with the risk of having schizophrenia, the 3 genotypes of G72 gene and its two component alleles, were found when compared to both control groups.

In conclusion, during the assessment of hypothalamic–pituitary–adrenal axis hormonal cascade only serum cortisol and adrenocorticotrophic hormone levels has a significant association with schizophrenia but not the human corticotropin releasing hormone serum level among schizophrenic patients. The significantly increased interleukin 6 in schizophrenic patient may enforce the pro inflammatory role in the disease pathogenesis, this is however not true for interleukin10. It was evident that this study revealed the significantly high serum levels of Neuregulin 1 protein in schizophrenic patients.

Genetic assessment of specific selected schizophrenia associated genes, Human leukocyte antigen -A*03 allele and B*40 allele considered as significant risk factors for schizophrenia, and the presence of high significant frequency of T allele and the heterozygous CT genotype in *Methylenetetrahydrofolate Reductase* gene indicates that C677T polymorphism is a risk factor for schizophrenia. In contrary the *D-Amino Acid Oxidase Activator* gene (G72) M18 polymorphism found to have no significant association with schizophrenia.

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List of Abbreviations

Abbreviation	Meaning
A	Adinine
ACTH	Adrenocorticotrophic hormone
AKT1	AKT serine/threonine kinase 1
ALSPAC	Avon Longitudinal Study of Parents and Children
APA	American Psychiatric Association
APCs	Antigen presentation complexes
APN	Aminopeptidase N
APOE	Apolipoprotein E
ARMS	At-risk mental state
BDNF	Brain derived neurotrophic factor
C	Cytocine
CD	Cluster differentiation
CHRNA7	Cholinergic receptor nicotinic alpha 7 subunit
CI	Confidence interval
CNS	Central nervous system
CNVs	Copy-number variations
COMT	catechol-O-methyltransferase
CRH	Corticotrophin releasing hormone
D ₂	Dopamine 2 receptors
DAO	D-amino acid oxidase
DAOA	D-Amino Acid Oxidase Activator
DISC1	Disrupted in schizophrenia 1
DLPFC	Dorsolateral prefrontal cortex
DNA	Deoxyribo Nucleic Acid
DOPA	<u>Dihydroxyphenylalanine</u>
DRD2	Dopamine receptor D2

DRD3	Dopamine receptor D3
DRD4	Dopamine receptor D4
DSM	Diagnostic and Statistical Manual of Mental Disorders
DTNBP1	Dystrobrevin binding protein 1
EAAC	Excitatory amino acid carrier
EGF	Epidermal Growth Factor
ELISA	Enzyme –Linked Immunosorbent Assay
ErbB4	Receptor tyrosine-protein kinase 4
GABA	Gamma-Aminobutyric acid
GBD	Global burden of disease
GR	Glucocorticoid receptor
GRM3	Glutamate metabotropic receptor 3
GWAS	Genome-wide association studies
H3N2	Influenza A virus subtype H3N2
HLA	Human leukocyte antigen
HPA	Hypothalamic pituitary adrenal
HTR2A	5-hydroxytryptamine receptor 2A
ICD	International Statistical Classification of Diseases
IDO1	Indoleamine-pyrrole 2,3-dioxygenase
IFN	Interferon
IL	Interleukin
IL10	Interleukin 10
IL-1 β	Interleukin 1 Beta
IL6	Interleukin 6
KCNN3	potassium calcium-activated channel subfamily N member 3
KYNA	kynurenic acid
MHC	Major histocompatibility complex
MTHFR	Methylenetetrahydrofolate Reductase
NMDA	N-methyl-D-aspartate

NMDAR	N-methyl-D-aspartate receptor
NOTCH4	Neurogenic locus notch homolog 4
NRG 1	Neuregulin 1
OR	Odds Ratio
PCR	Polymerase Chain Reaction
PF	Protective Fraction
PFC	prefrontal cortex
PGC	Psychiatric Genetics Consortium
PPP3CC	Protein phosphatase 3 catalytic subunit gamma
PRODH	Proline dehydrogenase 1
RFLP	Restriction Fragment Length polymorphism
RGS4	Regulator of G-protein signaling 4
SD	Standard Deviation
SE	Standard Error
SLC6A3	Solute carrier family 6 member 3
SLC6A4	Solute carrier family 6 member 4
SNP	Single nucleotide polymorphism
SNPs	Single Nucleotide Polymorphisms
SNS	Sympathetic nervous system
SPSS	Statistical Package for Social Sciences
SZ	Schizophrenia
T	Thymine
TBE	Tris-boric acid –EDTA
TGFβ	Transforming growth factor beta
Th	T helper cells
TNF-α	Tumor Necrosis Factor-α
UK	United Kingdom
USA	United States of America
UV	Ultra-Violet
WHO	World health organization