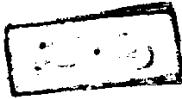


TOTAL HOMOCYSTEINE NEW TRENDS IN METHODS AND CLINICAL APPLICATIONS

ESSAY
Submitted in Partial Fulfillment of
Master Degree
in
Clinical and Chemical Pathology

By
Omaima Mohamed Taha
M.B., B.Ch.



53359

Supervisors

Dr. Nadia Ali Abdel-Sattar
Assistant Professor of Clinical Pathology
Faculty of Medicine
Ain Shams University

616 0756
C. M.

Handwritten signature or initials.

Dr. Ola Hamdy Demerdash
Assistant Professor of Clinical Pathology
Faculty of Medicine
Ain Shams University

Handwritten signature or initials.

Dr. Nashwa Ahmed Adel El Badawi
Assistant Professor of Clinical Pathology
Faculty of Medicine
Ain Shams University

Handwritten signature.

FACULTY OF MEDICINE
AIN SHAMS UNIVERSITY
1995





Acknowledgment

First and foremost, thanks are due to ALLAH

I would like to express my deep appreciation and gratitude to Prof. Dr. Nadia Abdel-Sattar, Assistant Professor of Clinical Pathology, Faculty of Medicine, Ain Shams University, for her scientific guidance, and continuous assistance during this work.

I shall always remain greatly indebted and much obliged to Dr. Ola Demerdash, Assistant Professor of Clinical Pathology, Faculty of Medicine, Ain Shams University for her kindness, giving much of her valuable time, her encouragement gave me a push to this work.

I would also like to express my deep appreciation to Dr. Nashwa El-Badawi, Assistant Professor of Clinical Pathology, Faculty of Medicine, Ain Shams University, for her valuable advice and help.

CONTENTS		Page
* INTRODUCTION AND AIM OF THE ESSAY		1
* REVIEW OF LITERATURE:		3
I- HISTORICAL VIEW		3
II - BIOCHEMISTRY OF HOMOCYSTEINE		4
III - REFERENCE VALUES & PHYSIOLOGICAL VARIATIONS OF TOTAL HOMOCYSTEINE		8
IV- METABOLISM OF HOMOCYSTEINE		11
A- Anabolism		11
B- Catabolism		11
C- Regulation of Various Pathways of Metabolism		12
D- Homocysteine Export to extracellular medium		15
E- Homocysteine Excretion		16
F- Methionine Loading		16
V- HOMOCYSTEINE ASSAY		18
A. Preanalytical Aspects of Homocysteine Assay.		18
B- Analytical Aspects of Homocysteine Assay.		22
VI- HOMOCYSTEINE AS A MARKER OF VARIOUS DISEASE STATES		45
A- Total Homocysteine in Cobalamin Deficiency.		45
B- Total Homocysteine in Folate Deficiency.		49
C- Total Homocysteine and Atherosclerosis.		53
D- Total Homocysteine in Renal Diseases.		58
E- Total Homocysteine in Down's Syndrom..		59
VII- HOMOCYSTINURIA		61
A- Homozygous Homocystinuria.		61
B- Heterozygous Homocystinuria.		70
VIII- MODULATORS OF PLASMA HOMOCYSTEINE		73
A. Folic Acid.		73
B. Methotrexate		73
C. Nitrous Oxide		75
D. Penicillamine.		75
E. Anticonvulsants.		76
F. Estrogen and Agents Affecting Estrogen Status.		77
G. Ethanol.		78
* Summary		80
* References		82
* Arabic Summary		----

LIST OF TABLES

No.	Title	Page
1	Normal values for total Hcy in human plasma	10
2	Stability of total Hcy in whole blood and plasma.	21
3	Construction of assays for total Hcy in plasma.	43
4	Characteristics, instrument requirements and performance of 5 methods for determination of total homocysteine in plasma.	44
5	Agents modulating the amount of plasma homocysteine in nonhomocystinurics	77

LIST OF FIGURES

No.	Title	Page
1	Molecular forms of homocysteine	4
2	Structure and distribution of different forms of homocysteine in human plasma	6
3	Scheme for the dynamic relation between various species of homocysteine in human plasma.	7
4	Frequency distribution curve for total plasma Hcy.	9
5	Homocysteine metabolism.	13
6	Various pathways involved in the metabolism of homocysteine.	14
7	Methionine loading of isolated rat hepatocytes.	17
8	Fluorogenic thiol (RSH)-specific reagents.	25
9	Functional drawing of a high-performance liquid chromatograph.	26
10	Illustration of thin layer chromatography.	31
11	Block diagram of the essential components of a mass spectrometer.	33
12	Determination of Hcy and other thiols in human plasma by automated precolumn derivatization with SBD-F.	35
13	Determination of Hcy and other thiols in human plasma by automated precolumn derivatization with mBrB.	36
14	Determination of Hcy in human plasma by HPLC and electrochemical detection.	38
15	A simplified flow diagram showing the basic components used in the ion-exchange chromatography.	40
16	Determination of Hcy in human plasma with an amino acid analyzer.	41
17	The relation between total Hcy and serum cobalamin.	47
18	The relation between total Hcy and serum folate.	51
19	Sites of deficiency in different forms of homocystinuria.	64

LIST OF ABBREVIATIONS

ABD-F	4 aminosulfonyl-7-fluoro-2,1,3-benzoxadiazole-4-sulfonate.
AgNO₃	silver nitrate
B₆	pyridoxine
B₁₂	cobalamin
EDTA	ethylenediaminetetra-acetate
GC	gas chromatography
Hcy	homocysteine
HPLC	high performance liquid chromatography
LDL	low density lipoprotein
mBrB	monobromobimane
MS	mass spectrometry
NaBH₄	sodium borohydride.
NaCN	sodium cyanide.
NaOH	sodium hydroxide.
NH₄OH	ammonium hydroxide.
SBD-F	ammonium-7-fluoro-2,1,3-benzoxadiazole-4-sulfonate.
SD	standard deviation.
TM	thrombomodulin.
V	labile factor.
Va	activated labile factor.
X	Staurt-Prower factor.
Xa	activated Staurt-Prower factor.

ABSTRACT

Omaima Mohamed Taha, Total homocysteine: new trends in methods and clinical applications.

**Clinical and Chemical Pathology Department
Faculty of Medicine, Ain Shams University,
1995.**

This review of literature aims to point out total homocysteine as regards its chemistry, metabolism, methods of assay, and clinical evaluation of total homocysteine as a marker of various disease states with emphasis on folate and cobalamin deficiencies, premature vascular diseases, and homocystinuria.

Homocysteine, a sulfhydryl amino acid, is the demethylated derivative of methionine. Hcy is a branch-point metabolite, the biologic fate of which is linked to vitamin B₁₂, reduced folates, and vitamin B₆.

Hcy in plasma forms mixed disulfides with other sulfur compounds and albumin. Total plasma Hcy includes all these forms. Plasma Hcy should be determined as such to avoid errors related to redistribution between different forms. Plasma Hcy shows a transient increase after methionine intake, and the postloading Hcy seems to be a measure of the efficiency of Hcy metabolism.

Several new Hcy methods have been developed and old techniques have been refined. The practical aspects of the techniques are quite different. Choice of a method depends on the personnel and instrumental resources available, the training of the technical staff, the anticipated number of samples to be analyzed, and special interests concerning the codetermination of other metabolites.

