Transforming Growth Factor–beta 1 in Patients with Hepatic Schistosomiasis

THESIS

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By

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List of Abbreviations

Ab	antibody
ADP	Adenosine Diphosphate
Ag	antigen
ALP	Alkaline Phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BSA	Bovine Serum Albumin
CD	Cluster of Differentiation
dl	deciliter
DNA	deoxyribo nucleic acid
ECM	Extracellular matrix
ELISA	Enzyme linked immuno sorbent assay
GAGs	Glycosaminoglycans
H_2O_2	Hydrogen peroxide
H_2O	water
HBs Ag	Hepatitis B surface antigen
HCV	Hepatitis C Virus
HDL	High density lipoprotein
HLA	Human Leucocyte antigen
HRP	Horseradish Peroxidase
I	Iodine
Ig	Immunoglobulin
kda	Kilo Dalton
Kg	Kilo gram
L	litre
LAP	Latency-associated peptide
LDH	Lactate Dehydrogenase
LDL	Low density Lipoprotein
M	mol
m^2	Square metre
MAD	Mothers against decapentaplegic gene products
MDH	Malate Dehydrogenase
mg	milli gram
MHC	Major Histocompatibility Complex
mmol	milli mol
mRNA	messenger ribonucleotide
NAD	Nicotinamide Adenine Dinucleotide (oxidized)
NADH	Nicotinamide Adenine Dinucleotide (reduced)
NaN_3	Sodium azide
ng	nano gram

NK	Natural Killer cells
nm	nano meter
NSB	Non-specific Binding
O_2	Oxygen
^oC	degrees Celsius
OD	optical density
PAI-1	Plasminogen activator inhibitor-1
PBS	Phosphate Buffered Saline
PCIIIP	Procollagen III peptide
pg	pico gram
PO_4	Phosphate
PPF	Periportal fibrosis
rpm	revolutions per minute
S.	Schistosoma
SHF	Schistosomal hepatic fibrosis
TG	Triglycerides
TGF-β	Transforming Growth Factor-Beta
TGF-β1	Transforming Growth Factor-beta1
Th	Thymocytes
TIMP	Tissue inhibitor of metalloproteinase
TMB	Tetra Methyl benzidine
U	units
VLDL	Very low density lipoprotein
α	alpha
Δ	change with time
λ	wave length
μg	micro gram

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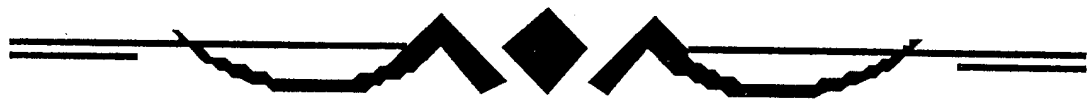

INTRODUCTION

CHAPTER 1

TRANSFORMING GROWTH FACTOR-BETA

Growth factors are polypeptides produced by cells that act to stimulate or inhibit proliferation of either the same cells or other cells. Several types of growth factors have been isolated and some of these may be associated with abnormal regulation of growth in transformed cells. Growth factors interact with cells through specific receptors in the cell membrane.[1]

Transforming growth factor-beta1 (TGF-β1) represents a large family of factors with diverse activities. The concept that TGF-β is prototypic of a super-family of growth, differentiation, and morphogenesis factors became clear in 1987.[2,3,4] Following the rich harvest that yielded the inhibins, activins, Müllerian inhibiting substance, decapentaplegic products, and TGF-β2, one after another, all these factors proved to be structurally related to TGF-β.[2,3,4] This family now includes embryogenic morphogenes, regulators of endocrine function and specialized regulators of cell proliferation and differentiation. The distribution of TGF-β-related factors is wide spread in living organisms. [5]

1. Structure:

The structural prototype for this gene super-family is the protein that was first isolated from human platelets as TGF-β.[6] TGF-β1 is a disulfide-linked 25 kDa dimer of two identical chains of 112 amino acids. Each chain is synthesized as the C-terminal domain of a 390 amino acids biologically inactive precursor that has the characteristics of a secretory polypeptide; it contains a hydrophobic signal sequence for translocation

across the endoplasmic reticulum and is glycosylated.[7,8,9] The precursor cleavage site is a sequence of four basic amino acids immediately preceding the bioactive domain.[10]

2. TGF-β isoforms:

There are three main isoforms of TGF-β: TGF-β1,TGF-β2, and TGF-β3. Each isoform is encoded by a distinct gene and is expressed in both a tissue-specific and a developmentally regulated fashion. TGF-β1 messenger RNA (mRNA) is expressed in endothelial, hematopoietic, and connective-tissue cells. TGF-β2 mRNA is expressed in epithelial and neuronal cells; and TGF-β3 mRNA is expressed primarily in mesenchymal cells. During development, TGF-β1 and TGF-β3 are expressed early in structures undergoing morphogenesis, while TGF-β2 is expressed later in mature and differentiating epithelium.[11]

The precursor structure is shared by all known members of the super-family with the exception of the TGF-β4 precursor, which lacks a discernable signal sequence.[10]

All three isoforms are highly conserved in mammals, suggesting a critical biologic function for each isoform. These isoforms differ in their binding affinity for TGF-β receptors.[12]

TGF-β1 is an important member of Transforming Growth Factor – Beta Family.

3. Formation:

TGF-β1 synthesis is limited in vivo to well defined cell populations. In humans, TGF-β is mainly produced by platelets, active monocytes and macrophages.[6]

Each TGF- isoform is synthesized as part of a large precursor molecule containing a propeptide region in addition to TGF-β. The cleavage of TGF-β from the propeptide occurs before the precursor is secreted by the cell but remains attached to the propeptide by noncovalent bonds. After it has been secreted, most TGF-β is stored in the extracellular matrix as a complex, consisting of TGF-β, the propeptide, and a protein called latent TGF-β-binding protein. The attachment of TGF-β to the binding protein occurs by disulfide bonds, which prevents it from binding to its receptors. There are four latent TGF-β-binding proteins; they are encoded by distinct genes and are expressed in a tissue-specific fashion.[12] (Figure.1).

4. Activation:

TGF-β is secreted from cells in a non-covalent complex between the latency-associated peptide (LAP) and the C-terminal dimer, which renders it biologically inactive or latent.(Fig.1) Activation of TGF-β occurs after extracellular dissociation of the mature form from the LAP. Transient acidification, proteolysis or chaotropic agents can cause this dissociation in vitro. The physiological activation of TGF-β1 is not well understood but plasmin and thrombospondin might be crucial.[9,13]

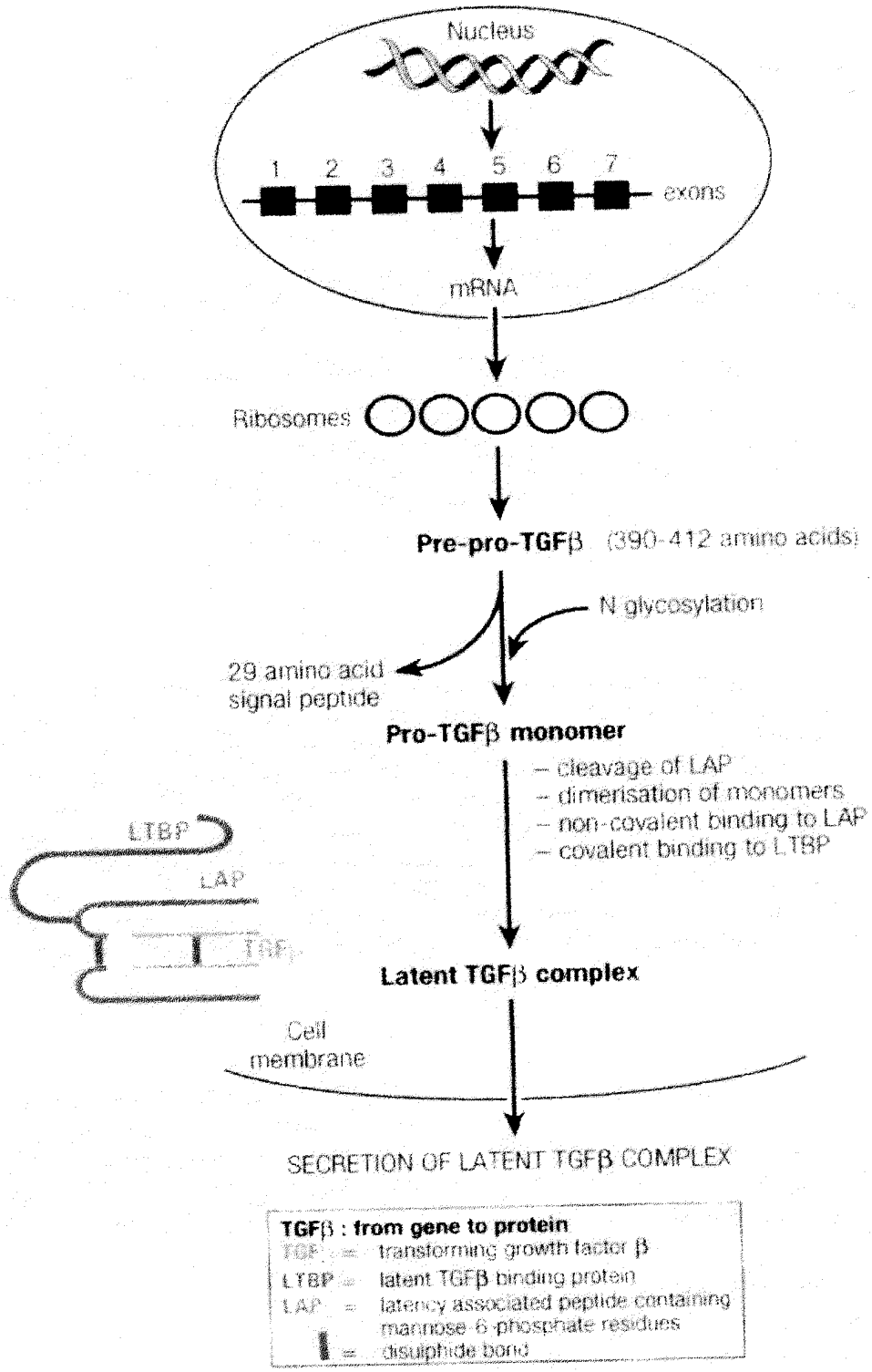

Fig. 1 Synthesis and secretion of TGF-β: From gene to released product. Details of intracellular processing is shown for TGF-β1. [14]

TGF-β is released from the complex by the multifunctional matrix glycoprotein thrombospondin-1, which appears to act by changing the conformation of the latent TGF-β-binding protein.[15] TGF-β may also be activated by plasmin- mediated cleavage of the complex. Since TGF-β and its receptors are present in most cells, this activation is probably a critical regulatory step in the action of TGF-β, which could limit biological action of TGF-β in particular cell types under specific conditions.[16,17] A notable exception to this process occurs in platelets, in which TGF-β is stored in intracellular granules that are released on platelet activation.[14,16](Figure.2).

5. Receptors:

TGF-β regulates cellular processes by binding to three high-affinity cell-surface receptors known as types I (55 kDa), II (80kDa), and III (280 kDa), which is the most abundant type.[16-19] (Fig. 2).

The type III receptor binds to TGF-β and then transferring it to its signaling receptors, the type I and II receptors. The nonsignaling role of type III receptors is shared by other abundant proteoglycan cytokine receptors, including syndecan for fibroblast growth factor, for nerve growth factor, and the type II receptor for insulin-like growth factor.[20]

The type I and II receptors contain serine-threonine protein kinases in their intracellular domains that initiate intracellular signaling. This occurs by phosphorylating several transcription factors known as Smads[19] (derived from the Sma and MAD gene homologues in *Caenorhabditis elegans* and *Drosophila melanogaste*r).[21] (Fig. 2 and 3).

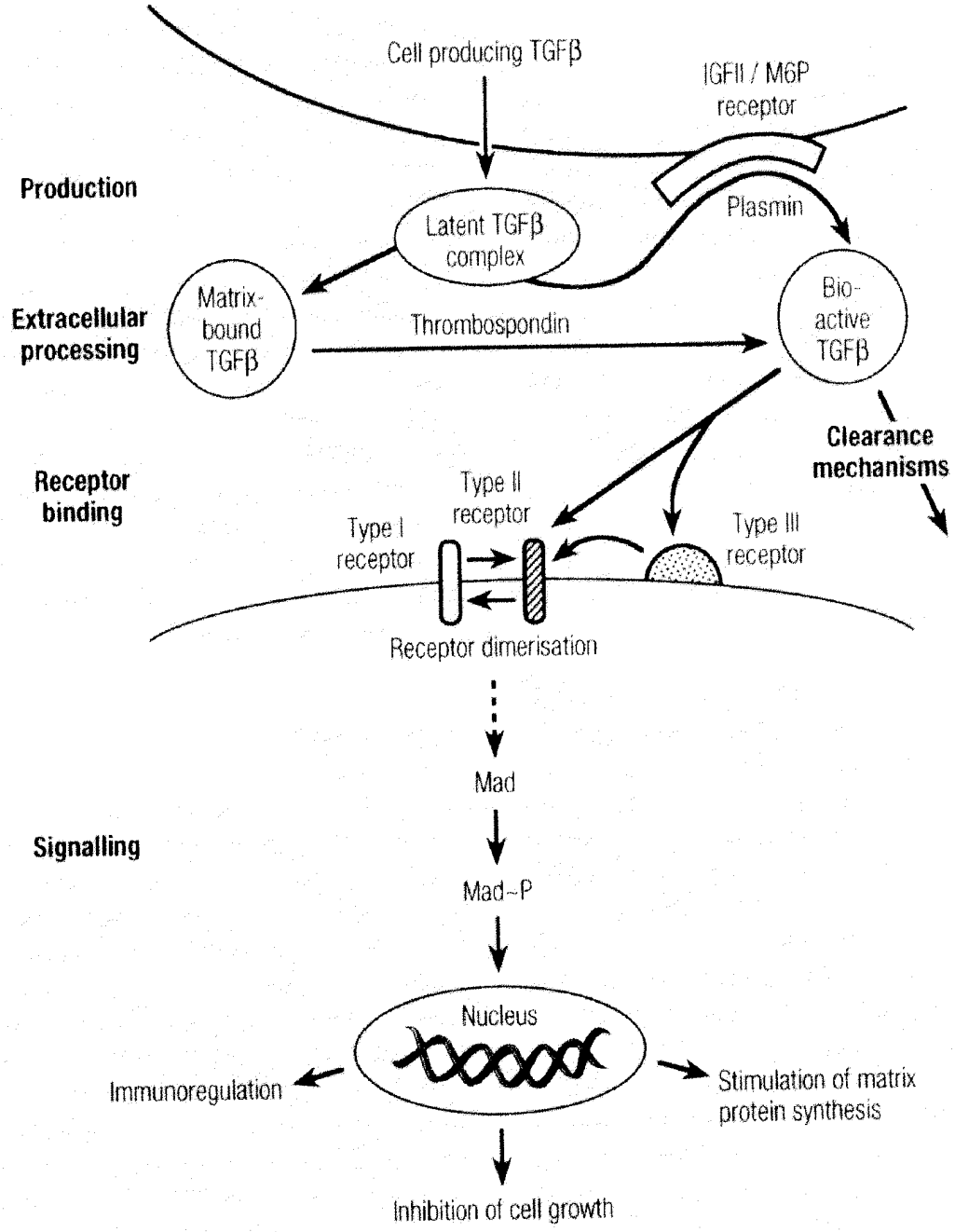

Fig. 2 Extracellular pathways of TGF-β: From release of latent form to action on receptors and catabolism.[14]

To date, 10 Smad proteins have been identified (Smads 1 through 10). Smad2 and Smad3 are phosphorylated by the activated type I receptor of TGF-β. Smad4 is a common partner for all of the receptor-activated Smads. Smad6 and Smad7 block the phosphorylation of Smad2 or Smad3, thus inhibiting TGF-β signaling.[21]

TGF-β binds either to type III receptors, which then present it to type II receptors, or TGF-β directly binds to type II receptors. Once Type II receptors are activated by TGF-β, they recruit, bind, and transphosphorylate the type I receptors, thereby stimulating their protein kinase activity. Following phosphorylation of Smad, the resulting Smad complex moves into the nucleus, where it interacts in a cell-specific manner with various transcriptional factors to regulate the transcription of many genes.[22-25]

There is also another TGF-β receptor called endoglin that is abundant on endothelial cells. It contains a transmembrane region and a cytoplasmic tail homologous to the type III receptor. Endoglin is mutated in patients with hereditary hemorrhagic telangiectasia.[26-28] TGF-β also acts through the mitogen-activated or stress-activated protein kinase pathways. [21]

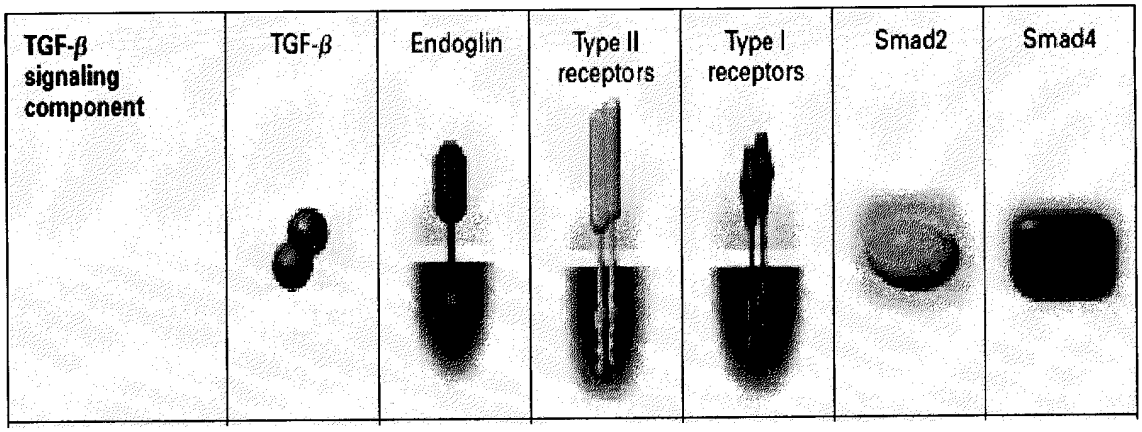

Figure. 3. Signaling components of TGF-β[29]

6. Biological actions of TGF-β:

The ability of TGF-β to elicit multiple cellular responses has been a subject of great interest. The paradigm of TGF-β as a dual factor, emanated first from studies on cell proliferation, can either inhibit or stimulate proliferation depending on the conditions.[22-25] The biological actions of TGF-β include:

a. *Cell-cycle regulation and effects on proliferation*:

In a dividing cell population, cell proliferation passes through a cycle that is divided into four phases, namely, G1 phase, S phase, G2 phase and M phase. The replication and division of a cell into genetically identical daughter cells depends on two functional phases and two preparatory phases (Figure 4). The functional phases of the cell cycle are the precise copying of the DNA, known as S phase or DNA replication, and the accurate segregation of duplicated sets of chromosomes between daughter cells, the M phase of mitosis. The cell prepares itself biochemically for S phase in a preparatory phase known as G1 (Gap 1) and prepares for mitosis in a poorly understood preparatory phase known as G2 (Gap 2)[30].

Cells that are not actively dividing may be either permanently removed from this cycling phase by terminal differentiation or be temporarily arrested in a non-cycling state known as G0. These events occur in an orderly fashion, with the requirement that some events in the cell cycle be completed before others begin.[30]

TGF-β regulates cellular proliferation in a cell-specific manner. In most epithelial, endothelial, and hematopoietic cells, TGF-β is a potent inhibitor of cell proliferation. It arrests the cell cycle in the G1 phase by stimulating the production of the cyclin-dependent protein kinase

inhibitor. It also does so by inhibiting the function or production of essential cell-cycle regulators.[31,32]

All TGF-β forms display reversible growth inhibitory activity in normal cells as well as in transformed epithelial, endothelial, fibroblast, neuronal, lymphoid and haematopoietic cell types.[22,24,25,31,33-42]

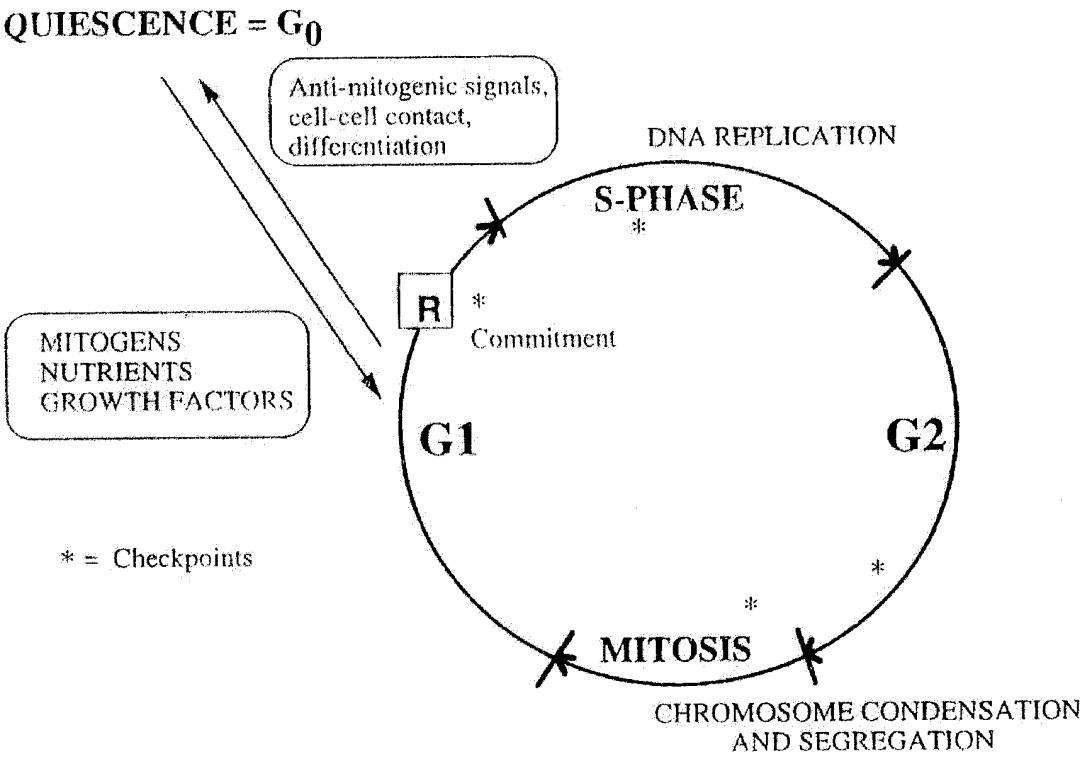

Figure 4. Schematic representation of cell cycle phases. Once a cell passes the restriction point ®, it is commited to progress through S phase.[43]

b. Control of cell adhesion:

Cell adhesion can guide cell migration, homing, and settlement during tissue formation, repair, tumor invasion, and metastasis by a complex set of adhesive interactions between cells and extracellular

matrices. Many of the cell surface components mediate adhesion of cells to the extracellular matrix and to other cells. The adhesive behaviour of a cell is determined in part by the type and level of adhesive receptors that it expresses, and also by the type of extracellular matrix that it produces and with which it interfaces. In addition to their physical support effect, adhesive interactions are a major conduit for intercellular regulation of cell function and phenotype. The cell adhesion apparatus and the composition of extracellular matrices might be regulated by growth and differentiation factors. This is clearly apparent in the action of TGF-β on many cell types.[44]

The action of TGF-β on normal mesenchymal, epithelial, and lymphoid cells, as well as various tumor cell lines, generally leads to up-regulation of cell adhesion. This action is mediated in concert by enhanced synthesis and deposition of extracellular matrix components, decreased pericellular proteolysis, and modification of the repertoire of cell surface adhesion receptors.[44]

c. Control of extracellular matrix protein expression:

The action of TGF-β elevates fibronectin expression in several mesenchymal and epithelial cell types, both normal and transformed.[45,46] Up to tenfold elevation in fibronectin synthesis are frequently observed in response to TGF-β1. This is followed by a corresponding accumulation of extracellular matrix fibronectin.[47,48] TGF-β also regulates the expression of type I collagen α1 and α2 chains, as well as collagen types III, VI, and X.[45,49,50] In addition, expression of type II collagen is induced in mesenchymal muscle cells secondary to their chondrogenic differentiation in response to TGF-β1.[51]

d. Control of pericellular proteolysis:

The increased synthesis of extracellular matrix components induced by TGF-β is not solely responsible for the net accumulation of extracellular matrix. Plasminogen activator inhibitor-1 (PAI-1) and tissue inhibitor of metalloproteinase (TIMP), which are inhibitors of extracellular matrix degrading enzymes, are also strongly up-regulated by TGF-β.[52-54] The up-regulation of PAI-1 mRNA is due, at least in part, to increased transcription, that occurs with faster kinetics (<30 min) than the response of other extracellular matrix components, and can reach up to 50-fold over the basal level. In addition, TGF-β can also decrease the expression of collagenase,[55] transin/stromelysin,[56,57] plasminogen activator,[52] and a thiol proteinase.[58]

7. Degradation:

There are many postulations for the degradation of TGF's-β. Bioactive TGF's-β may bind to matrix, to α_2-macroglobulin and to decorin. This binding usually inhibits their activity. They may also bind to carriers such as albumin and newly synthesized IgG, but this binding does not inhibit their activity. Alpha$_2$-macroglobulin may facilitate the action of TGF-β on cells bearing α_2-macroglobulin receptors. TGF-β-α_2-macroglobulin complexes are taken up via hepatic mannose-6-phosphate or insulin like growth factor II receptors, possibly to be catabolized.[59] Bioactive TGF-β's may also be degraded by proteases and elastases released at sites of inflammation or it may be excreted in urine.[14]

Role of TGF-β in disease

Role of TGF-β in wound healing and tissue repair:

TGF-β isoforms can play a central role in wound healing and in tissue repair. TGF-β is produced or released by infiltrating cells such as lymphocytes, monocytes, macrophages and platelets. Following a wound or inflammation, all these cells are potential sources of TGF-β. In general, release and activation of TGF-β stimulates the production of various extracellular matrix proteins and inhibits the degradation of these matrix proteins. Both these actions contribute to tissue repair, which under ideal circumstances leads to the restoration of normal tissue architecture and may also be involved in a component of tissue fibrosis.[60]

Role of TGF-β in atherosclerosis:

TGF-β inhibits the proliferation and migration of smooth muscle and endothelial cells. It was suggested that TGF-β could function as an inhibitor of atherosclerosis. Consistent with this hypothesis are the findings that serum levels of TGF-β are low in patients with atherosclerosis and that tamoxifen therapy may mediate its cardioprotective effects by increasing serum TGF-β levels.[61-63] In addition, mutations or decreased expression of the type II receptor gene has been detected in clonal populations of cells of atherosclerotic lesions. These alterations in the type II receptor render the cells resistant to the antiproliferative and apoptotic effects of TGF-β. This suggests that genetic alterations in the TGF-pathway contribute to this prevalent disease.[63,64]

Role of TGF-β in glomerular injury: (e.g. Diabetic nephropathy and hypertensive glomerular injury)

A possible explanation for the kidney's susceptibility to fibrosis may be the discovery of biologically complex interactions between the renin-angiotensin system and TGF-β. Alteration in glomerular hemodynamics can activate renin-angiotensin system, TGF-β and plasminogen activator inhibitor leading to rapid matrix accumulation. The protective effect of inhibition of renin-angiotensin system correlates with the suppression of TGF-β production. In volume depletion, TGF-β acts synergistically with angiotensin II accentuating vasoconstriction and acute renal failure.[65]

Role of TGF-β in malignancy:

In cancer cells, mutations in the TGF-β pathway have been described that confer resistance to growth inhibitory action of TGF-β, thus allowing uncontrolled proliferation of the cells.[31]

The extent of the growth inhibitory response to TGF-β varies with the cell type. TGF-β acts by lengthening or arresting the G1 phase of the cell cycle.[66-70] It reaches a virtual growth arrest in certain lung epithelial cells, lung fibroblasts and keratinocytes.[22,36,71]

The production and secretion of TGF-β by certain cancer cells suppress the activities of infiltrating immune cells, thereby helping the tumor escape host immunosurveillance.[72] TGF-β can also directly stimulate angiogenesis in vivo. The TGF-β antibodies can block this stimulation.[73] Expression of the TGF-β receptor endoglin, is greatly increased during angiogenesis.[74] Both the immunosuppressive effect and

the stimulatory effect of angiogenesis may be other mechanisms by which TGF-β stimulates the growth of late-stage tumors. It can also increase the invasiveness of the cells by increasing their proteolytic activity and promoting their binding to cell-adhesion molecules.[75]

TGF-β and the liver

Cellular origin of TGF-β in the liver:

In the liver, synthesis of TGF-β involves non-parenchymal cells, mainly Ito cells in the space of Dissë, Kupffer cells and endothelial cells.[76-79] Whether hepatocytes can produce TGF-β1 is subject to debate. Immunohistochemical studies and *in-situ* hybridization for TGF-β1 do not show any staining in hepatocytes of normal liver. In cirrhotic human liver, hepatocytes displays a significant intracellular expression of TGF-β1 at the protein and mRNA levels.[79-82] In cirrhotic tissues, co-localization of TGF-β1 with the extracellular matrix is also observed, suggesting that some extracellular matrix proteins can bind this cytokine. This accumulation might either protect TGF-β1 from degradation and function as a local reservoir of cytokine, or act as a clearance system.[83]

Hepatic cell control and proliferation:

In the liver, TGF-β1 blocks hepatocytes division and inhibits Ito cell proliferation during liver fibrogenesis.[84] The mechanism underlying the inhibitory effect of TGF-β1 on hepatic cell proliferation has been characterized. TGF-β1 can induce dephosphorylation of the retinoblastoma gene product, which might inhibit the entry of cells into the S phase due to its affinity for DNA.[85,86]

TGF-β in liver regeneration and liver fibrogenesis:

TGF-β has been shown to be a promotor of extracellular matrix deposition and a regulator of cell migration, suggesting that it probably plays a major role in liver regeneration in chronic liver diseases.[87] There is a transient increase in TGF-β mRNA levels in the regenerative liver following partial hepatectomy. This is followed by a transient increase in extracellular matrix proteins.[87] When tissue repair is complete, an unknown mechanism inhibits the TGF-β expression and the ensuing matrix production.[88]

Failure to inhibit expression of TGF-β or its sustained stimulation may lead to TGF-β autostimulation and pathologic matrix accumulation, hence liver fibrosis occurs.[89,90] The evidence that TGF-β plays a critical role in these conditions was initially correlative. TGF-β1 mRNA and extracellular matrix deposits have been detected in serum and liver tissue in a number of hepatic fibrosis models including schistosomiasis infection.[89,90] In humans, an increase in serum TGF-β1 has been found in chronic active hepatitis, in correlation with the histological activity index, collagen mRNA production and serum Pro-collagen III peptide (PCIIIP) levels.[89,90] An increase of TGF-β1 has been also found in active cirrhosis of various origins.[91]

TGF-β1 effect on Ito cells and extracellular matrix production:

Studies have consistently shown that TGF-β accelerates the transformation of resting Ito cells to myofibroblasts.[84,92] Whether TGF-β

induces directly Ito cells multiplication has not been clarified and requires further studies.[84,93] However, it is known that TGF-β induces the expression of other cytokines and their receptors by Ito cells. This, in turn, stimulates Ito cell proliferation.[92,93]

TGF-β increases the production of many extracellular matrix proteins in fibroblasts or Ito cells culture. Accumulation of fibronectin, type I, III and VI collagens, tenascin, elastin, osteonectine, thrombospondin, biglycan and decorin are observed after TGF-β stimulation of fibroblasts or Ito cells.[5,94,95] Increased levels of corresponding mRNA are observed within hours after TGF-β is added and are the consequence of both increased gene transcription and mRNA stabilization.[96] TGF-β also upregulates the fixation, elongation and termination of glycosaminoglycans (GAGs) to core proteins of proteoglycans.[97] In addition to regulating genes encoding extracellular matrix components, TGF-β enhances its own expression by an autocrine regulatory mechanism at transcriptional and post-transcriptional levels.[98] Thus the net effect of TGF-β is an increase in extracellular matrix accumulation, a possible shift in the extracellular matrix composition, and autocrine stimulation of TGF-β expression.[99]

TGF-β effects on extracellular matrix degradation:

The TGF-β induced accumulation of extracellular matrix proteins is not solely related to an increase in extracellular matrix production. TGF-β can also downregulate the production of collagenase, stromelysin, elastase, plasminogen activator and other proteinases involved in the destruction of extracellular matrix.[100]

TGF-β also upregulates plasminogen activator inhibitor (PAI-1) and tissue inhibitor of metalloproteinase (TIMP) by endothelial and Ito cells. These two enzymes can inhibit the degrading enzymes of extracellular matrix proteins.[98,100]

CHAPTER 2

HEPATIC SCHISTOSOMIASIS

[I] Normal hepatic matrices

(A) Normal hepatic cellular matrix:

The liver contains various different cells, each with distinct functions. Classically, the liver cells are divided into parenchymal cells (hepatocytes) and nonparenchymal cells. These latter cell types include endothelial cells, Kupffer cells, hepatic stellate cells and pit cells. Each type of cell has evolved special characteristics that allow the cells to perform key roles both in normal liver function and liver injury.[101]

Sinusoidal endothelial cells allows rapid and full exchange between the blood and liver cells., they have an important and selective role, in removing denatured and damaged proteins before they are presented to hepatocytes.[102,103]

Kupffer cells are liver macrophages, they are currently described as cells that have phagocytic activity and are located along the lining of the sinusoid. They are markedly decreased in cirrhosis, and this may lead to the increased susceptibility to infections in cirrhosis due to bacteria in the portal vein.[104,105]

Hepatic stellate cells have been proposed to regulate sinusoidal blood flow both in a normal or injured liver. The resting stellate cell produces small amounts of collagen types III and IV, while the activated cell produces primarily collagen type I but can also produce collagen III, IV,

VI, fibronectin, hyaluronic acid and other proteins.. This occurs regardless of the type of injury and has been documented in many liver diseases.[106]

Pit cells are the most recently recognized group of nonparenchymal cells, also known as the natural killer (NK) cells. They are found within the sinusoidal lumen. They are nonphagocytic and do not have any endogenous peroxidase activity. It has been documented that there is an accumulation of pit cells in viral hepatitis.[105]

Ito cells are the resting precursor of fibroblasts in the space of Dissë. Typical fibroblasts are found only in portal tracts.[107]

In normal liver, hepatocytes, fat producing cells and endothelial cells produce the extracellular matrix.[108]

(B) Normal hepatic extracellular matrix:

The extracellular matrix (ECM) in the liver is formed of collagens type I and III,[109,110] proteoglycans,[111-113] laminin,[114-116] fibronectin[117,118] and tenascin.[119] The precise pathways that direct and regulate the synthesis of collagen and other matrix proteins have not been fully elucidated.
A shift in the usual balance of matrix degradation and synthesis leads to fibrosis.[120]

I. Collagen is a heterogenous class of extracellular proteins characterized by a unique amino acid composition. It consists of about 30% glycine, 20% proline, hydroxyproline and a variable content of hydroxylysine. There are 19 homopolymeric or heteropolymeric collagen types. They all share a triple-helical segment of variable length (100 to 450 mm). The central feature of all collagen molecules is the stiff structure resulting from lengthy domains of triple-helical conformation. Three polypeptide

chains called alpha-chains are turned around one another to generate a rope-like fold. An absolute requirement for the formation of this triple helix, as well as the most distinctive feature of the alpha-chains, is the presence of lengthy sequences of repeating Gly-X-Y triplets in which the X and Y positions are frequently occupied by prolyl and hydroxyprolyl residues.[121] (Figure.5) But the collagens differ considerably in the size and nature of their globular domains. There are 3 types of collagen in the liver (table 1.)

Table 1. The types of collagen in the liver.[122]

Type	Site	Stained by
I	Portal zones, central zones, broad scars	Van Giesen
III	Reticulin fibres (sinusoids, portal zones)	Silver
IV	Basement membranes	Periodic acid Schiff (PAS)

Myofibroblasts in Dissë space may similarly produce collagen.[107]

Aminoterminal *procollagen III peptide* (PCIIIP) is cleaved off the procollagen molecule in the synthesis of collagen type III fibril. Its level in serum is not of practical diagnostic value, but it is useful in monitoring the degree of liver fibrosis.[123,124] However, in chronic liver disease increased levels may reflect inflammation and necrosis rather than fibrosis.[125]

II. Fibronectin is a cell-surface glycoprotein, which mediates the attachment of collagen fibrils and proteoglycans to hepatocytes. It is formed by endothelial cells in the space of Dissë.[126] It also modulates cell differentiation and function, particularly during the healing process.

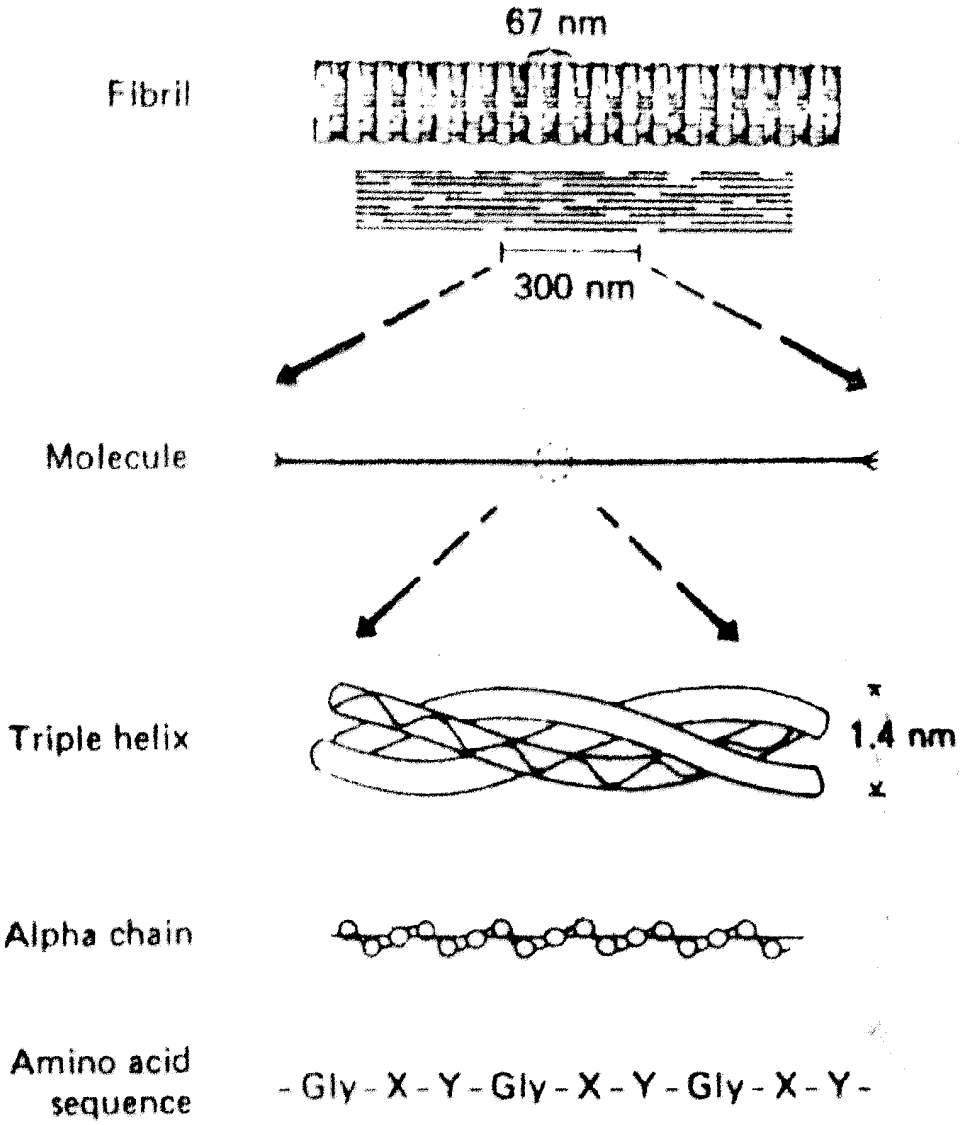

Figure.5. Molecular features of collagen structure from primary sequence up to the fibril.[127]

III. Laminin is a large, rigid glycoprotein produced by Ito cells and endothelial cells. It forms a continuous layer around hepatic sinusoids adjacent to hepatocytes and sinusoidal lining cells.[114] It is also present in basement membranes of ducts, ductules and capillaries. It forms a basement membrane around hepatocytes during regeneration or hepatic injury.[128]

IV. Elastin is an insoluble protein polymer. The tropoelastin, which is the biosynthetic precursor of elastin is a linear polypeptide composed of about 700 amino acids. It is rich in non-polar amino acids: glycine (>30%), valine, leucine, isoleucine, and alanine.[121]

The proteins and metabolites of connective tissue metabolism spillover into the plasma where they can be measured. Unfortunately, they reflect fibrosis generally and do not give information specifically about hepatic fibrosis.[123,124]

[II] Fibrogenesis

Fibrogenesis is preceded by expansion of Ito cells,[107] which alter their collagen gene expression, so that they become phenotypically more fibroblast-like.[107]

Pathological fibrosis represents excessive or disordered production of hepatic extracellular matrix,[129] which contains collagen I, III and IV, fibronectin, large glycoproteins and proteoglycans.[130] Fibrosis is not simply a passive structural support for the damaged hepatocytes but may modulate their behaviour.[131] In fibrosis, hepatocytes which normally do not synthesize type III and type IV collagen may produce them.[108]

Together with collagen, fibronectin is deposited in areas of liver cell damage within one hour of the injury. Fibronectin stimulates fibroblasts and its breakdown products are chemotactic for fibroblasts.[121]

[III] Hepatic schistosomiasis

Schistosomiasis is the most common parasitic infection in Egypt. It is caused by trematode blood flukes of the genus Schistosoma. Schistosoma (S.) haematobium and S. mansoni are the most common types responsible for the infection in Egypt.[132]

After penetration of the skin of exposed persons, the cercariae of these worms circulate as schistosomules via the lymphatic and blood vessels. Finally they reach the mesenteric venous plexus where they get mature, differentiate into males and females, mate together and start to ovipose. This occurs within 6 to 8 weeks from the primary exposure.[133,134]

Shortly after developing into schistosomulae, the parasites shed surface antigens. [135] They develop glycolipids similar to those of blood groups [136] and develop also major histocompatibility complex (MHC) antigens from the host.[137] By expressing host antigens on their tegument, the schistosomes resemble the host's tissue and thereby avoid attack by the immune system.[138]

Schistosome eggs remaining near the mesenteric venous plexus elicit an intense granulomatous response that can lead to inflammatory or ulcerative lesions of the intestinal wall. Alternatively, eggs are released into the portal circulation and are carried by blood flow into the liver, where they lodge in the small portal vein tributaries. [132]

Although the host suffers directly little damage from the adult worms, the eggs elicit an intense granulomatous inflammatory response. The egg granulomas are well-circumscribed aggregates of inflammatory cells composed of eosinophils, lymphocytes, macrophages, and neutrophils embedded in a collagenized extracellular matrix.[139] The formation of hepatic granuloma in *S. mansoni* infections is mediated by major histocompatability complex (MHC) class II-restricted $CD4^+$ Thymocytes (Th) cells.[138]

Granuloma formation in the liver leads to pyelophlebitis, peripyelophlebitis, and then periportal fibrosis. Histopathologic examination of the hepatic lesions of chronic schistosomiasis reveals portal fibrosis with partial or complete destruction of the main branches of the portal vein, although the arterial and ductal structures are preserved. Despite the intense fibrotic changes, the lobular architecture of the hepatic parenchyma usually is maintained. [132] The ultra structure of hepatocytes is minimally affected. [140]

The fibrous tissue in hepatic fibrosis is composed of collagen types I, III, IV, and V, procollagen type III, actin, desmin, fibronectin, and laminin.[141] This pattern of scarring, often referred to as Symmers' pipe stem fibrosis because of the clay pipe stem appearance on gross pathology.[142] In more severe cases, this leads to the development of portal hypertension and associated complications.[135] The collagen deposition at the sinusoidal surface of the hepatocytes will lead to blocking of this surface, which may affect its function of exchange of metabolites and oxygen. This may be the explanation why late cases of hepatic schistosomiasis will show definite impairment of hepatic functions, which are relatively spared, in early and moderate cases.[140]

Hepatic schistosomiasis varies greatly in severity and case presentation. Many cases have mild illness or are even asymptomatic and remain so for many years. While others have advanced fibrotic shrunken livers with portal hypertension, splenomegaly, oesophageal varices, ascites or encephalopathy. This variation is related to the intensity of worm load and/or the duration of infection. However it seems that there are other factors that may determine the morbidity of hepatic schistosomiasis. Variations of strains in different countries, nutritional status of the victims, concomitant other infections, prevalence of hepatotoxins in the community, immunological state and genetic distinction of individual patients (HLA typing), were occasionally accused. [143]

The degree of fibrosis produced by haematobium worms is claimed to be less than that produced by mansoni worms which are known to be more fibrogenic. This is simply due to an inherited difference between the two worms.[144]

[IV] Association of hepatic schistosomiasis and hepatitis C viral infection

Patients with schistosomiasis have higher rates of hepatitis C seropositivity than do non-infected subjects. [145] The prevalence of hepatitis C virus (HCV) infections in schistosomal patients ranges from 14.3-75.5%. [146] In Egypt, a study revealed that the prevalence of HCV antibodies was 43.3% among them. [147] This association is believed to be,

at least partly, due to transmission of hepatitis viruses during blood transfusion and/or the parenteral therapy for schistosomiasis with contaminated needles. [148] Schistosomiasis potentiates HCV infections by prolonging hepatic inflammation after an episode of acute viral hepatitis and by increasing the risk of chronic infection. [148,149]

The association of schistosomiasis with HCV infection makes the pathological, clinical, biochemical and hemodynamic pictures different. There may be early evidence of hepatic cell dysfunction in the form of jaundice, edema of the lower limbs, ascites,..etc. The surface of the liver will be irregular due to the regenerative nodules. The biochemical indices of hepatic functions will show considerable deviation from normality. [140] A cirrhotic liver may contain several-fold more matrix than normal liver resulting in compromised hepatic function. [150]

[V] TGF-β and hepatic schistosomiasis

Cytokines control the host response to the S. mansoni ova from the very outset through its resolution. [151] They are essential for communication not only between the liver and extra-hepatic sites, but also within the liver itself. Since many cytokines exert growth-factor-like activities, in addition to their pro-inflammatory effects, the distinction between cytokines and growth factors is somewhat artificial. [152]

Local production of TGF-β contributes to schistosome-induced liver disease. It is particularly contributed to the accumulation of the extracellular matrix proteins. TGF-β mRNA and protein have been identified within the lesion, locally and temporally concordant with

deposition of collagen and other matrix constituents. [153-155] TGF-β is a well-known trigger for the synthesis of these molecules. [156-158] Hepatic production of TGF-β by liver cells and/or by infiltrating inflammatory cells may also regulate schistosome-induced liver fibrosis. [159-161]

In Schistosomiasis, some of the known atherogenic risk factors do not exist. Advanced schistosomal hepatic fibrosis was thought to be atheroprotective, since the incidence of atherosclerosis is not common in these patients [162]. There are many causes for this protection, where the associated hyperestrogenemia, [163,164] hypolipidemia [165] are the most important causes.

Atherosclerosis is associated with numerous risk factors, where hyperlipidaemia is one of them. [166] In schistosomal patients hypocholesterolaemia, [162,165,167-172] with low level of LDL-Cholesterol and normal HDL-Cholesterol levels were found. [172,173]

Hyperestrogenaemia [164,174] is also common in schistosomiasis. Oestrogen is known to protect against atherosclerosis and ischemic heart disease.[175] In addition, the presence of hypocoagulability and hyperfibrinolysis in schistosomal patients may contribute for the diminished tendency to thrombo-vascular lesions. [176]

In atherosclerosis, there is an alteration of the endothelial barrier by some factors. One of these factors is the TGF-β, which is a potent inhibitor of smooth muscle cell mitosis. It also stimulates the elaboration of matrix proteins such as fibronectin and vascular collagen [121] and its activation is done by trypsin-like serine proteases, such as plasmin. [18] It has been reported that TGF-β is diminished in patients with atherosclerosis. [161]

AIM OF THE WORK

AIM OF THE WORK

The objective of this study was to estimate the serum level of transforming growth factor – beta1 in patients with schistosomal hepatic fibrosis, in an attempt to evaluate its fibropathogenic and atheroprotective roles.

MATERIAL

MATERIAL

Fifty-eight subjects were included in this study, their age ranged from 21 – 65 years.

They were categorized as follows:

- Group I:

  It included 20 healthy volunteers of comparable age, sex and socioeconomic status as patients.

- Group II:

  It included 18 patients with schistosomal hepatic fibrosis (SHF), who were negative for both hepatitis B surface antigen and anti-HCV antibodies.

- Group III:

  It included 20 patients with mixed infection of hepatic schistosomiasis and hepatitis C. All were negative for hepatitis B surface antigen.

Criteria of selection of the studied subjects was based on exclusion of conditions that might alter TGF-β1 level, such as malignancy, diabetes mellitus, myelofibrosis, systemic sclerosis, glomerulonephritis, and Crohn's disease. This was done by clinical and/or laboratory means.

All subjects were normotensive, non smokers and had normal fasting serum glucose levels.

METHODS

METHODS

To all subjects the following was done:

I. Thorough clinical examination including full history taking with special stress on schistosomal and hepatitis infection; weight, height, blood pressure, chest, heart and abdominal examination. All patients were previously diagnosed by laboratory means as schistosomal patients

II. **Calculation of body mass index (BMI):**[177] This was done by the following equation:

BMI = Weight in Kg/ Square of the height in meters.

The ideal BMI ranges from 18.5 - ≤25 Kg/m^2. Subjects who had BMI above 25 Kg/m^2 were considered obese.

III. **Investigations:**

(a) Clinical investigation:

1. Electrocardiography (ECG).
2. Plain X-ray on the chest.
3. Abdominal ultrasonography.

(b) Laboratory investigations:

Specimen preparation and storage:

After 12-14 hours of overnight fasting, 10 ml venous blood were withdrawn and centrifuged after clotting and the resulting serum was divided as follows:

- An aliquot of serum was used for the detection of Hepatitis B-surface antigen (HBs-Ag) and anti-Hepatitis C virus (HCV) antibodies, as well as for determination of glucose, bilirubin, enzyme activities and lipid pattern.
- An aliquot of serum was kept frozen at –70°C for estimation of procollagen III propeptide (PCIIIP).
- An aliquot of serum was kept frozen at –70°C for estimation of Transforming growth factor-beta1 (TGF-β1).

1. *Serodetection of HBs-Ag by sandwich enzyme linked immuno-sorbent assay (ELISA) technique:* [178]

The sample was incubated in microwells coated with polyclonal anti-HBs antibodies. Any HBs-Ag in sample would bind to immobilized anti-HBs Ab. Unbound material was removed by washing.

The formed Ag-Ab complex was then incubated with conjugate, anti-HBs/Biotin, which would bind to the complex, enzyme conjugate Streptavidin/POD was then added. Excess unbound enzyme conjugate was removed by washing.

The bound enzyme was detected by addition of a suitable substrate, Tetra Methyl Benzidine (TMB).

The colour which developed in wells, which contain HBs-Ag, was read photometrically. All readings above the cutoff value were considered positive and were excluded.

2. *Serodetection of anti-HCV antibodies by sandwich enzyme linked immuno-sorbent assay (ELISA) technique:* [179]

The sample was incubated in microwells coated with highly purified HCV Ag. Any anti-HCV Ab in sample would bind to immobilized HCV Ag. Unbound material was removed by washing.

The formed Ag-Ab complex was then incubated with peroxidase conjugated monoclonal anti-human IgG, which would bind to the complex. Excess unbound enzyme conjugate was removed by washing.

The bound enzyme was detected by addition of a suitable substrate. The colour which developed in wells, which contain anti-HCV Ab was read photometrically. All readings above the cutoff value were considered positive.

3. *Estimation of Fasting Glucose level:* [180]

Glucose present in the sample was determined without deproteinization by glucose oxidase method, where glucose was oxidized by the enzyme glucose oxidase in the presence of oxygen to gluconic acid with the formation of hydrogen peroxide. In a second reaction, the enzyme peroxidase catalyzed the oxidation of a chromogen oxygen acceptor by the hydrogen peroxide resulting in the formation of a colored product, which was proportional to the glucose present initially in the first reaction.

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{glucose oxidase}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{reduced chromogen} \xrightarrow{\text{peroxidase}} \text{Oxidized chromogen} + H_2O$$

The Resulted rosy colour was measured spectrophotometrically at λ 505nm after comparison with a standard glucose of known concentration similarly treated.

The concentration of glucose in the sample was obtained from the following equation:

$$\text{mg glucose/dl} = \frac{\text{absorbance of sample} \times \text{concentration of the standard}}{\text{absorbance of standard}}$$

$$\text{mmol glucose/L} = \text{mg/dl} \times 0.055$$

4. *Serum total bilirubin determination:* [180]

Dimethyl sulfoxide was used as a solvent for total bilirubin assay. Bilirubin in this solvent reacts with diazotized sulfanilic acid and produced an intensely coloured diazo dye. The intensity of the colour of this dye was proportional to the concentration of total bilirubin.

Absorbance of sample is read at λ 546 nm and the results were obtained after comparison with a standard bilirubin of known concentration similarly treated using the following equation:

$$\text{mg bilirubin/dl} = \frac{\text{absorbance of sample} \times \text{concentration of the standard}}{\text{absorbance of standard}}$$

5. *Serum direct bilirubin:* [180]

Direct bilirubin in sample reacted with diazotized sulfanilic acid to produce a diazo dye.

The intensity of colour of this dye was directly proportional to the concentration of direct bilirubin in the sample.

Absorbance of the sample was read at λ 546nm and the results were obtained after comparison with a standard bilirubin of known concentration similarly treated using the following equation:

$$\text{mg bilirubin/dl} = \frac{\text{absorbance of sample} \times \text{concentration of the standard}}{\text{absorbance of standard}}$$

6. *Estimation of Total serum cholesterol:*[181]

It was determined enzymatically according to the following reaction:

$$\text{Cholesterol-ester} \xrightarrow{\text{Cholesterol esterase}} \text{cholesterol + fatty acid}$$

$$\text{Cholesterol} \xrightarrow{\text{Cholesterol oxidase}} \text{Cholestene-3-one} + H_2O_2$$

$$2H_2O_2 + \text{phenol} + \text{4-aminoantipyrene} \xrightarrow{\text{peroxidase}} \text{quinoneimine} + H_2O$$

The rose coloured quinoneimine in the sample (T) was measured at λ 505nm and compared to the colour of a similarly treated standard (S) of a known cholesterol concentration (Cs). The concentration of cholesterol was obtained by the following equation:

mg cholesterol/dl = T/S x Cs

(mmol cholesterol/L = mg/dl x 0.026)

7. *Estimation of High density lipoprotein (HDL) cholesterol:*[180]

It was determined enzymatically after precipitation of low density lipoproteins (LDL and VLDL) and chylomicrons by the addition of phosphotungestic acid in the presence of magnesium ions. The cholesterol concentration of HDL left in the supernatent after centrifugation was measured enzymatically as in the above method.

8. *Estimation of Low density lipoproteins (LDL) cholesterol:*[180]

It was determined enzymatically after precipitation of LDL-cholesterol fraction by LDL precipitating mixture (polycyclic anionic

surfactant, and polysubstituted dioxane in appropriate pH buffer). The LDL pellet precipited after centrifugation was solubilized by LDL solubilizing reagent (trisodium citrate and sodium chloride) and its cholesterol content was measured enzymatically.

9. *Triglycerides (TG) estimation:*[182]

It was determined enzymatically without deproteinization according to the following reactions:

Enzymatic hydrolysis with lipase:

$$TG \xrightarrow{\text{lipase}} \text{Glycerol + fatty acids}$$

$$\text{Glycerol + ATP} \xrightarrow{\text{glycerol kinase}} \text{glycerol-3 } PO_4 + \text{ADP}$$

$$\text{Glycerol-3 } PO_4 + O_2 \xrightarrow{\text{glycerol-3 PO4 oxidase}} \text{dihydroxyacetone } PO_4 + H_2O_2$$

Colour development:

$$2H_2O_2 + \text{parachlorophenol} + \text{4-Aminoantipyrene} \xrightarrow{\text{peroxidase}} \text{quinoneimine (red coloured)} + 4H_20$$

The rosy coloured chromogen which is proportional to TG concentration in the sample (T) was measured spectrophotometrically at λ 505nm and compared to standard of known TG concentration (Cs) similarly treated (S). TG concentration was obtained by the following equation:

mg TG/dl = T/S x Cs

(mmol/L triglycerides = mg/dl x 0.0145)

10. *Determination of Serum aspartate aminotransferase (AST) activity:*[180]

This was done kinetically using the following reactions:

$$\text{L-aspartate + 2-oxoglutarate} \xleftrightarrow{\text{AST}} \text{L-Glutamate + Oxaloacetate}$$

$$\text{Oxaloacetate} + \text{NADH} + H^+ \xleftrightarrow{\text{MDH}} \text{L-malate} + NAD^+$$

The resulting decrease in absorbance at λ 340 nm of NADH at 37°C, was followed with time (ΔT) and it was directly proportional to the activity of AST in the sample.

Calculation: AST activity (U/L) = ΔA/min x 1481

11. *Determination of Serum alanine aminotransferase (ALT) activity:* [180]

This was done kinetically using the following reactions:

$$\text{L-alanine} + \text{2-oxoglutarate} \xleftrightarrow{\text{ALT}} \text{L-Glutamate} + \text{Pyruvate}$$

$$\text{Pyruvate} + \text{NADH} + H^+ \xleftrightarrow{\text{LDH}} \text{D-Lactate} + NAD^+$$

The resulting decrease in absorbance at λ 340 nm of NADH at 37°C, was followed with time (ΔT) and it was directly proportional to the activity of ALT in the sample.

Calculation: ALT activity (U/L) = ΔA/min x 1481

12. *Serum Alkaline Phosphatase (ALP) activity:* [180]

This was done kinetically using the following reaction:

$$\text{p-nitrophenylphosphate} + H_2O \xleftrightarrow{\text{ALP}} \text{Phosphate} + \text{p-nitrophenol}$$

The resulting increase in absorbance of p-nitrophenol at λ 405 nm was followed with time (ΔT) and it was directly proportional to the activity of ALP in the sample.

Calculation: ALP activity (U/L) = ΔA/min x 2757

13. *Procollagen III Peptide (PCIIIP) estimation:* [183]

This was estimated using the intact PCIIIP assay kit, which was based on the competitive radio-immunoassay technique (Type III Procollagen Intact Radioimmunoassay Kit, manufactured by Orion Diagnostica, Finland).

The unknown amount of PCIIIP in the samples was mixed with a known amount of a radioactively labeled derivative of PCIIIP antigen. The labeled and the unlabelled antigens were allowed to compete for the limited number of high affinity binding sites of the anti-PCIIIP antibodies. The amount of radioactive antigens in the antigen-antibody complex was inversely proportional to the amount of unlabelled antigen in the sample. After separating the free antigen from the antibody-antigen complex, the residual radioactivity was counted by gamma counter and the actual concentration was calculated with the aid of a standard curve based on known amounts of unlabelled antigen analysed in parallel with the unknown.

The results of standard count/mean zero-standard count (B/B0) were plotted using semi-log graph paper, which produced a sigmoid curve.

Reagents:

1. PCIII [^{125}I] reagent:

   Ready to use solution of ^{125}I-labelled PCIIIP in PBS (Phosphate Buffered Solution) buffer with BSA (Bovine Serum Albumin), a red colour additive and 0.05% NaN_3 as a preservative.

2. PCIIIP antiserum:

   Ready to use solution of PCIIIP antiserum (rabbit) in PBS buffer with BSA, a blue colour additive and 0.05% NaN_3 as a preservative.

3. PCIIIP standards: (7 vials)

Ready to use preparations of human serum containing <0.1% NaN_3 0. 1.0, 2.5, 5.0, 10, 25 and 50 µg PCIIIP/L, respectively.

4. PCIIIP control:

Lyophilised control of human serum containing <0.1 NaN_3 as a preservative. The expected value was indicated on the separate result sheet provided with the kit.

Reconstitution of the control serum:

The vial was allowed to reach equilibrium at room temperature before it was opened. It was reconstituted by adding 1.5 ml distilled water to the vial.

The control vial has been capped, mixed well by gentle swirling or inversion to avoid foaming. It was then allowed to stand for 10 minutes before it was used.

5. Procollagen separation reagent:

A ready to use suspension of a second antibody covalently bounded to solid particles, with <0.1% NaN_3 as a peservative.

Procedure:

1. All reagents, control and samples were brought to room temperature.
2. A series of tubes in duplicate were labeled for NSB (non-specific binding), PCIIIP standards, controls, patient samples and total counts.
3. 200µl of each of standards, control or patient samples were pipetted into a corresponding set of tubes. Into the NSB tubes, 200µl of any patient sample was pipetted.
4. 200µl of PCIIIP [^{125}I] reagent (red) were pipetted into all tubes.

5. Then 200µl of PCIIIP antiserum solution (blue) were pipetted into all tubes except the tubes of NSB and total counts. 200µl of distilled water were pipetted into NSB tubes.
6. All tubes were mixed well, capped and covered then incubated for 2 hours at 37°C.
7. The separation reagent was mixed thoroughly by gentle inversion and 500µl were added to all tubes except that of the total counts.
8. The tube contents were thoroughly mixed using a vortex mixer and left to stand for 30 minutes at room temperature.
9. The tubes were then centrifuged at 2000 g for 15 minutes at 20°C.
10. The tubes were placed carefully into decanting racks to decant the supernatant, the mouth of the tubes were dried by tapping against absorbent paper.
11. The radioactivity was counted using gamma counter for 1 minute per tube or until 10 000 count per tube have accumulated.
12. The final PCIIIP concentrations were determined by interpolation from the standard curve.

Calculation:

The results were derived using standard curve produced on semi-log graph paper.

1. The mean count for NSB tubes were calculated and subtracted from all standards, control and unknown tube counts.
2. The mean of the NSB corrected counts from the zero standard tubes were calculated.

3. The (B/B0)% were calculated from :

$$(B/B0)\% = \frac{(\text{Standard, control or sample count} - \text{NSB}) \times 100}{(\text{Mean zero standard count} - \text{NSB})}$$

4. A standard curve was drawn on semi-log graph paper with (B/B0)% on the ordinate and PCIIIP concentrations in (μg/L) of the standards on the abscissa.
5. The PCIIIP concentrations of the unknowns were read and the duplicates were averaged.

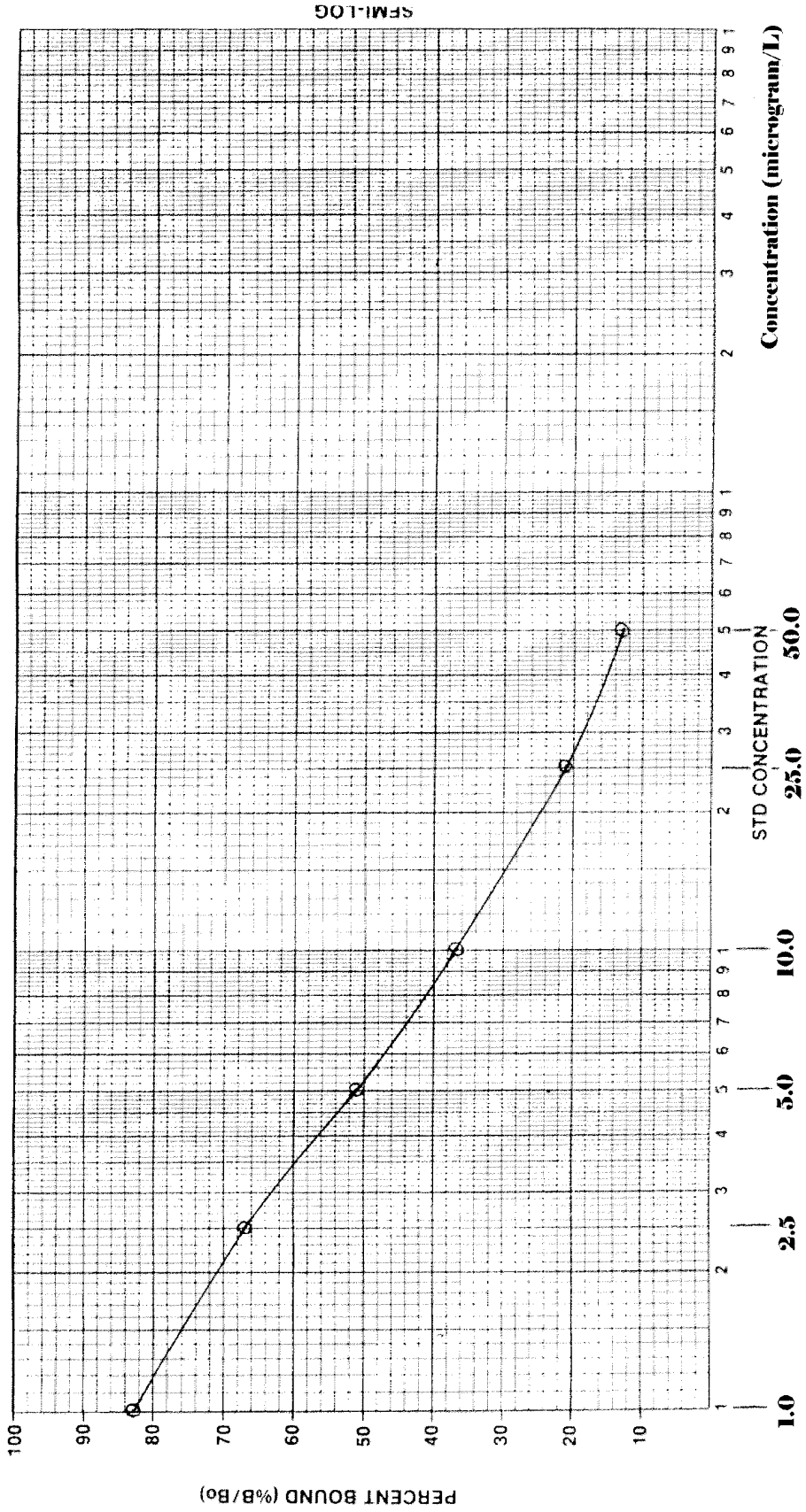

Figure.6: Standard curve for Pro-collagen III peptide

14. *Estimation of Transforming Growth factor-Beta (TGF-β1) by ELISA:*[184]

This was done by competitive enzyme immunoassay, using TGF-β1 ELISA kit (Manufactured by BioSource Europa S.A. in Belgium. Catalogue number KAC1681). A fixed amount of TGF-β1 labeled with horseradish peroxidase (HRP) competes with unlabeled TGF-β1 present in standard or extracted samples for a limited number of binding sites of specific coated antibodies on the plate wells. After 2 hours incubation at room temperature with continuous shaking, the microtiter plate is washed to stop the competition reaction. The chromogenic solution, tetra methyl benzidine (TMB) is added and incubated for 30 minutes. The reaction is stopped with the addition of Stop Solution. The final colour was read at 450 nm using an ELISA reader. The amount of substrate turnover is determined colorimetrically by measuring the absorbance, which is inversely proportional to the TGF-β1 concentration. A similarly treated standard curve was plotted and TGF-β1 concentrations in samples were determined by interpolation from the standard curve.

Reagents:

1. Microtiter plate with 96 wells coated with anti-TGF-β1: ready for use.
2. Stock TGF-β1 standard (1000 pg/mL): One vial of the lyophilized TGF-β1 from human platelets in acetate buffer.
3. TGF-β1 control in human plasma.
4. TGF-β1 - HRP conjugate: ready for use.
5. Dilution buffer: ready for use.
6. Extraction solution: ready for use.

7. Acetic acid 2.5M: ready for use.
8. Chromogenic TMB solution (Tetramethylbenzidine) : ready for use.
9. Stop Solution: ready for use.
10. Washing Solution.

Reagent preparation:

1. Stock Standard solution for TGF-β1 (1000 pg/ml):
   One vial of lyophilized TGF-β1 standard was reconstituted with 0.5 ml dilution buffer (= the 1/1 calibrator, undiluted).
2. Serial standards preparation: In polypropylene tubes, a 5-fold serial dilutions of the reconstituted calibrator were performed with dilution buffer as in the table below:

Table 2. Serial standards preparation:

Standard dilutions	Volume of calibrator	Vol. of Dilution buffer added	Concentration
1/1 undiluted			1000pg/ml
1/5	0.2 ml calibrator 1/1	0.8 ml	200 pg/ml
1/25	0.2 ml calibrator 1/5	0.8 ml	40 pg/ml
1/125	0.2 ml calibrator 1/25	0.8 ml	8 pg/ml

3. Control (TGF-β1): The control was reconstituted with 0.5 ml distilled water.
4. Wash Solution: The content of the washing solution has been diluted in 2000 ml distilled water.

Control and Samples extraction:

This step has allowed for the release of TGF-β1 from latent complexes, making it accessible for measurement in the enzyme-immunoassay.

In a polypropylene tubes, 0.1 ml of each sample and control were added to 0.1 ml 2.5 M acetic acid. Then mixed by vortex and incubated for 15 minutes at room temperature. They were then diluted by adding 500 µl dilution buffer to 20 µl of the mixture in other polypropylene tubes.
By this extraction step, the samples were diluted 1/52.

Assay Procedure:

1. The required number of the coated wells in the strips were selected for the run.
2. The strips were secured into the holding frame.
3. 100 µl of each standard and extracted sample were pipetted into the appropriate wells.
4. 50 µl of TGF-β1-HRP conjugate were pipetted into all the wells.
5. The plate was incubated for 2 hours at room temperature on a horizontal shaker set at 700 rpm ±100.
6. The liquid which contained the unbound antigen was then aspirated from each well.
7. The plate was washed by: dispensing 0.4 ml of wash solution into each well, then aspirating the content of each well. This washing step was repeated for three times.
8. Then, 100 µl of chromogen (TMB) were pipetted into each well within 15 minutes following the washing step.

9. The plate was incubated for 30 minutes in the dark at room temperature on a horizontal shaker set at 700 rpm ± 100.
10. At the end of incubation, 100 µl stop solution were pipetted into each well.
11. The absorbances were then read at 450 nm (reference filter: 650 nm) within 3 hours and the results were calculated.

Calculation of analytical results:

$$\% \text{ of } B/B0 = \frac{OD \text{ (standard or samples)} \times 100}{OD \text{ (zero standard)}}$$

- The B/B0 x 100 was plotted using semi log graph paper, for each standard point after multiplication by the dilution factor of 52 in order to obtain the TGF-β1 concentrations.
- By interpolation of the samples (% of B/B0) values, TGF-β1 concentrations of the extracted samples were determined from the reference curve straightforwardly.

STATISTICAL ANALYSIS[185]

The data were analyszed by personal computer using the SPSS software for statistical analysis version 9, which included.

1. Arithmatic mean ($\overline{x}$)

It was calculated from the following formula:

$$\overline{x} = \frac{\sum x}{n}$$

Where $\sum x$ = Sum of observations.

n = Number of observations.

2. Standard deviation (S.D.)

It was calculated by the following formula:

$$S.D. = \sqrt{\frac{\sum x^2 - (\sum x)^2 / n}{n-1}}$$

Where: $\sum x^2$ = Sum of squared observations.

$(\sum x)^2$ = Square of the sum of observations.

3. Standard error (S.E.)

It was calculated by the following formula:

$$S.E. = \frac{S.D.}{\sqrt{n}}$$

4. Analysis of the differences

The differences of the mean ±S.D. of a variable between two or more groups were considered significant if the probability "p" value was less than 0.05. The following tests of significance were used to study the differences:-

a) When there were homogenous samples (S.D. <mean) or there was big sample size (≥10 samples)

Parametric analyses were used, which differ according to the number of groups present:

◊ If the comparison was done between two groups only, the student "t" test was used.

◊ If the comparison was done between more than two groups, the analysis of variance (ANOVA) was used.

b) When there were unhomogenous samples (S.D. ≥mean) or there was small sample size

Non-parametric analyses were used, which differ according to the number of groups present:

◊ If the comparison was done between two groups only, Mann-Whitney (Wilcoxon Rank test) was used. The level of significance was measured by "Z" test.

◊ If the comparison was done between more than two groups, Kruskal-Wallis test was used. The level of significance was measured by Chi-square test.

Student "t" test (parametric method)

It was used to compare between two sample means. It was calculated as follows:

$$t = \frac{x_1 - x_2}{\sqrt{(SE_1)^2 + (SE_2)^2}} \quad , \qquad df = (n_1+n_2)-2$$

Where

x_1 = Mean of the first group.

x_2 = Mean of the second group.

SE_1 = Standard error of the first group.

SE_2 = Standard error of the second group.

df = Degree of freedom.

Analysis of variance (parametric method)

It was used to compare more than two groups (three and four groups). The level of significance in this analysis of variance (ANOVA) was measured by "F" test. If it was significant, the comparison between the groups was done by the least significant difference (LSD).

Mann-Whitney (Wilcoxon Rank) test (Non-parametric method)

It was used to compare the differences between two independent groups, where the two groups were ranked together in an ascending order, and then each rank was averaged. The level of significance was measured by "Z" test.

Kruskal-Wallis test (Non-parametric method)

It was used to compare the differences between more than two groups, where all samples from the groups are combined and ranked together and their average was obtained. Also, for each group, the ranks are summed and squared. Then by computing a special formula, a Chi-square was used to measure the significance.

5. Pearson correlation

It was used to measure the strength of the relationship between two numerical variables. It was obtained from the following formula:

$$r = \frac{n(\sum xy) - (\sum x)(\sum y)}{\sqrt{[n(\sum x^2) - (\sum x)^2][n(\sum y^2) - (\sum y)^2]}}$$

Where n = The number of paired observations.

$\sum xy$ = The sum of all variables.

$\sum x$ = The sum of x-variables.

$\sum y$ = The sum of y-variables.

$\sum x^2$ = The sum of squared x-variables.

$\sum y^2$ = The sum of squared y-variables.

$(\sum x)^2$ = The square of the sum of x-variables.

$(\sum y)^2$ = The square of the sum of y-variables.

The "r" value ranged between –1.0 to +1.0.

RESULTS

Table I: Clinical data in the control group

The table shows some clinical data of the control group.

- The age ranged from 28-61 years with a mean of 37.6±9.64 years.
- They included 17 males (85%) and 3 females (15%).
- Body mass index ranged from 21-26 Kg/m^2 with a mean of 23.4±1.5 Kg/ m^2.

Table I: Clinical data in the control group

No.	Age (years)	Sex	BMI (Kg/m^2)
1	61	M	22
2	57	M	23
3	47	M	22
4	34	M	24
5	32	M	25
6	50	M	23
7	35	M	23
8	36	M	21
9	31	F	22
10	28	M	23
11	28	F	23
12	29	M	26
13	30	M	23
14	29	M	24
15	38	F	21
16	32	M	26
17	38	M	24
18	42	M	24
19	43	M	23
20	32	M	26
Mean ±S.D.	37.6 9.64		23.4 1.5

BMI = Body Mass Index
M = Male
F = Female
S.D. = Standard deviation

Table IIa: Clinical Data in SHF patients with negative anti-HCV antibodies

The table shows some clinical data of SHF patients with negative anti-HCV antibodies.

- The age ranged from 30-47 years with a mean of 37.8±5.77 years.
- They included 13 males (72.2%) and 5 females (27.8%).
- Body mass index ranged from 21-28 Kg/m^2 with a mean of 24.7±2.3 Kg/m^2.
- 61.1% had hepatomegaly (11 cases out of 18).
- 61.1% had mild splenomegaly (11 cases) and 38.9% had moderate splenomegaly (7 cases).
- 27.8% had ascites (5 cases out of 18).
- By ultrasonography, they all had periportal fibrosis of the liver, where 33.3% had grade I PPF, 38.9% had grade II PPF and 27.8% had grade III PPF.
- 38.9% (7 cases out of 18) had dilated portal vein (>13 mm).

Table IIa. Clinical Data in patients with negative anti-HCV antibodies

No.	Age (Years)	Sex	BMI (Kg/m^2)	Hepato.	Spleno.	Ascites	Lower Limb edema	Liver by U/S	Portal Vein by U/S
1	30	M	22	No	1	No	No	I	Normal
2	47	F	28	Yes	2	Yes	Yes	III	Dilated
3	37	M	22	Yes	2	No	Yes	II	Dilated
4	45	M	26	Yes	2	No	No	II	Dilated
5	35	M	21	Yes	1	No	No	II	Normal
6	31	M	23	No	1	No	No	I	Normal
7	35	M	23	No	1	No	No	I	Normal
8	39	M	26	No	1	No	No	II	Normal
9	31	M	24	No	1	No	No	I	Normal
10	45	M	27	Yes	2	Yes	Yes	III	Dilated
11	43	F	28	Yes	2	Yes	Yes	III	Dilated
12	30	F	27	Yes	1	No	No	II	Normal
13	38	M	23	No	1	No	No	I	Normal
14	47	F	28	Yes	2	Yes	Yes	III	Dilated
15	34	M	24	Yes	1	No	Yes	II	Normal
16	35	M	23	Yes	1	No	No	II	Normal
17	37	M	24	No	1	No	No	I	Normal
18	41	F	25	Yes	2	Yes	Yes	III	Dilated
Mean ±S.D.	37.8 5.77		24.7 2.3						

SHF = Schistosomal hepatic fibrosis
Spleno. = Splenomegaly
Hepato. = hepatomegaly

Liver by U/S: Grades of periportal fibrosis

Splenomegaly:
1 = Mild
2 = Moderate

M = Male
F = Female

S.D.= Standard deviation

Table IIb: Clinical Data in SHF patients with positive anti-HCV antibodies

The table shows some clinical data of SHF patients with positive anti-HCV antibodies.

- The age ranged from 36-65 years with a mean of 44.5±7.52 years.
- They included 16 males (80%) and 4 females (20%).
- Body mass index ranged from 21-39 Kg/m^2 with a mean of 24.8± 4.4 Kg/m^2.
- 65% had hepatomegaly (13 cases out of 20).
- 30% had mild splenomegaly (6 cases out of 20), 55% had moderate splenomegaly (11 cases out of 20), 10% had huge splenomegaly (2 cases out of 20) and 5% were splenectomized (1 case out of 20).
- 40% had ascites (8 cases out of 20).
- By ultrasonography, they all had mixed hepatic fibrosis and cirrhosis, where 45% had grade I PPF, 25% had grade II PPF and 30% had grade III PPF.
- 65% (13 cases out of 20) had dilated portal vein (>13mm).

Table IIb: Clinical Data in SHF patients with positive anti-HCV antibodies

No.	Age (Years)	Sex	BMI (Kg/m^2)	Hepato.	Spleno.	Ascites	Lower Limb edema	Liver by U/S	Portal Vein by U/S
1	38	F	33	Yes	2	No	Yes	I	Dilated
2	45	F	26	Yes	2	No	Yes	I	Dilated
3	50	M	23	Yes	2	Yes	Yes	II	Dilated
4	37	M	29	No	2	No	Yes	II	Dilated
5	65	F	39	No	2	Yes	Yes	II	Dilated
6	52	F	26	Yes	2	Yes	Yes	III	Dilated
7	30	M	21	Yes	2	No	Yes	III	Dilated
8	46	M	23	Yes	2	Yes	Yes	II	Dilated
9	41	M	22	No	2	No	Yes	I	Dilated
10	45	M	23	Yes	1	No	No	I	Normal
11	44	M	24	Yes	1	No	No	I	Normal
12	46	M	22	Yes	1	No	Yes	I	Normal
13	49	M	23	Yes	3	Yes	Yes	III	Dilated
14	48	M	21	No	1	No	No	II	Normal
15	36	M	25	No	2	No	Yes	I	Dilated
16	36	M	24	Yes	1	No	No	I	Normal
17	40	M	26	Yes	2	Yes	Yes	III	Dilated
18	51	M	23	No	3	Yes	Yes	III	Dilated
19	45	M	21	No	1	No	Yes	I	Normal
20	46	M	22	Yes	0	Yes	Yes	III	Normal
Mean ±S.D.	44.5 7.52		24.8 4.4						

SHF = Schistosomal hepatic fibrosis

Spleno. = Splenomegaly
Hepato. = hepatomegaly

Liver by U/S: Grades of periportal fibrosis

Splenomegaly:
0 = Splenectomized
1 = Mild
2 = Moderate
3 = Huge

M = Male
F = Female

S.D. = Standard deviation

Table III: Statistical analysis of clinical data in the three studied groups

The table shows the body mass index in the three studied groups and their statistical significances.

It also contains the age and the sex distribution among the studied groups.

Table III: Statistical analysis of clinical data in the three studied groups

Group / Variable	Controls	SHF Patients	
		-ve anti HCV Abs	+ve anti HCV Abs
Age (years)			
Mean	37.6	37.8	44.5
±S.D.	9.64	5.77	7.52
Sex (number)			
Males	17 (85%)	13 (72.2%)	16 (80%)
Females	3 (15%)	5 (27.8%)	4 (20%)
BMI (kg/m2)			
Mean	23.4	24.7	24.8
±S.D.	1.5	2.3	4.4
P1		N. S.	N. S
P2			N. S.

SHF = Schistosomal hepatic fibrosis

S.D. = Standard deviation

P1 = Statistical significance between controls and each of the patient groups.
P2 = Statistical significance between both patients groups.

Table IV: Some serum enzymes and total bilirubin in the control group

The table shows some serum enzymes and total bilirubin in the control group.

- The AST values ranged from 13-40 U/L with a mean of 21.6±6.54 U/L.
- The ALT values ranged from 6-40 U/L with a mean of 22.5±11.1 U/L.
- The ALP ranged from 78-201 U/L with a mean of 132.45±36.47 U/L.
- The total bilirubin values ranged from 0.4-1 mg/dL with a mean of 0.73±0.19 mg/dL.

Table IV: Some serum enzymes and total bilirubin in the control group

No.	AST (U/L)	ALT (U/L)	ALP (U/L)	Total Bilirubin (mg/dL)
1	15	17	158	0.5
2	13	12	201	0.8
3	16	19	133	0.4
4	18	23	153	0.7
5	21	6	112	0.9
6	17	21	112	0.7
7	25	23	140	0.7
8	34	40	141	0.5
9	20	11	96	0.5
10	22	15	95	0.8
11	16	9	95	0.5
12	22	33	105	0.9
13	28	34	133	1.0
14	40	31	78	0.8
15	21	12	105	0.9
16	25	40	193	0.9
17	20	31	141	1.0
18	20	29	193	0.9
19	23	36	168	0.7
20	16	8	97	0.5
Mean ±S.D.	21.6 6.54	22.5 11.1	132.45 36.47	0.73 0.19

AST = Aspartate amino transferase
ALT = Alanine amino transferase
ALP = Alkaline phosphatase

S.D. = Standard deviation

Table Va: Some serum enzymes and bilirubin in SHF patients with negative anti-HCV antibodies

The table shows some serum enzymes and bilirubin in the SHF negative anti-HCV antibodies patients group.

- The AST values ranged from 15-70 U/L with a mean of 30.17±15.29 U/L.
- The ALT values ranged from 12-149 U/L with a mean of 36.89±32.17 U/L.
- The ALP values ranged from 90-559 U/L with a mean of 236.88±122.37 U/L.
- The total bilirubin values ranged from 0.5-2.5 mg/dL with a mean of 1.1±0.548 mg/dL.
- The direct bilirubin values ranged from 0.1-1.0 mg/dL with a mean of 0.47±0.27 mg/dL..

Table Va: Some serum enzymes and bilirubin in SHF patients with negative anti-HCV antibodies

No.	AST (U/L))	ALT (U/L)	ALP (U/L)	Bilirubin (mg/dL)	
				T. Bil.	D. Bil.
1	27	23	183	1.1	0.7
2	52	46	359	0.8	0.3
3	23	26	115	2.2	0.6
4	40	149	375	0.9	0.3
5	25	17	90	2.5	0.6
6	16	24	120	1.2	1.0
7	22	34	200	0.6	0.2
8	24	44	278	0.5	0.2
9	21	23	128	0.7	0.1
10	19	23	---	1.6	1.0
11	25	21	305	1.3	0.5
12	39	33	167	0.5	0.2
13	20	44	139	0.9	0.3
14	29	13	265	1.4	0.8
15	56	35	559	0.9	0.4
16	20	12	272	0.9	0.5
17	15	18	156	1.1	0.4
18	70	79	316	0.7	0.3
Mean ±S.D.	30.17 15.29	36.9 32.17	236.88 122.37	1.1 0.548	0.47 0.27

SHF = Schistosomal hepatic fibrosis

AST = Aspartate amino transferase
ALT = Alanine amino transferase
ALP = Alkaline phosphatase

T. Bil. = Total bilirubin
D. Bil. = Direct bilirubin

S.D. = Standard deviation

Table Vb: Some serum enzymes and bilirubin in SHF patients with positive anti-HCV antibodies

The table shows some serum enzymes and bilirubin in the positive anti-HCV antibodies group.

- The AST values ranged from 23-132 U/L with a mean of 57.6±30.58 U/L.
- The ALT values ranged from 8-142 U/L with a mean of 49.4±41.89 U/L.
- The ALP values ranged from 97-420 U/L with a mean of 228.35±91.29 U/L.
- The total bilirubin values ranged from 0.5-3.2 mg/dL with a mean of 1.39±0.83 mg/dL.
- The direct bilirubin values ranged from 0.2-1.6 mg/dL with a mean of 0.60±0.49 mg/dL.

Table Vb: Some serum enzymes and bilirubin in SHF patients with positive anti-HCV antibodies

No.	AST (U/L)	ALT (U/L)	ALP (U/L)	Bilirubin (mg/dL)	
				T. Bil.	D. Bil.
1	38	24	270	0.7	0.2
2	66	18	177	1.7	0.8
3	34	29	397	0.5	0.2
4	35	36	157	0.9	0.3
5	47	8	131	---	---
6	32	9	246	---	---
7	81	142	230	1.6	0.6
8	132	128	195	1.6	0.8
9	74	117	245	0.5	0.2
10	26	37	149	1.8	0.2
11	50	30	280	0.9	0.2
12	40	27	176	0.8	0.2
13	101	85	420	3.2	1.3
14	36	41	358	2	1.2
15	26	22	132	0.7	0.2
16	23	32	97	1.0	0.6
17	77	29	257	2.8	1.6
18	82	30	290	0.9	0.3
19	48	32	116	2.7	1.5
20	104	112	244	0.8	0.4
Mean ±S.D.	57.6 30.58	49.4 41.89	228.35 91.29	1.39 0.83	0.6 0.49

SHF = Schistosomal hepatic fibrosis
AST = Aspartate amino transferase
ALT = Alanine amino transferase
ALP = Alkaline phosphatase

T. Bil. = Total bilirubin
D. Bil. = Direct bilirubin

S.D. = Standard deviation

Table VIa: Statistical significance in the serum enzymes between the control group and whole SHF patients group

The whole patients group had significantly higher activities of ALT, AST and ALP than the control group ($p < 0.05$, < 0.001, < 0.001; respectively)

Table VIa: Statistical significance in the serum enzymes between the control group and whole SHF patients group

Group / Variable	Controls	SHF Patients
Number	20	38
ALT (Units/L)		
Mean	22.5	43.47
±S.D.	11.1	37.64
p		<0.05
AST (Units/L)		
Mean	21.6	44.61
±S.D.	6.54	27.93
p		<0.001
ALP (Units/L)		
Mean	132.45	232.27
±S.D.	36.47	105.23
p		<0.001

SHF = Schistosomal hepatic fibrosis
AST = Aspartate aminotransferase
ALT = Alanine aminotransferase
ALP = Alkaline phosphatase

S.D.= Standard deviation

p : Statistical significance between control group and whole patients group.

Table VIb: Statistical significance in the serum enzymes and total bilirubin between the controls and SHF patients groups

- The positive anti-HCV patients group showed significantly higher AST values than that in both the control and the negative anti-HCV antibodies groups (P1, 2 <0.05).
- The positive anti-HCV patients group showed significantly higher ALT values than that in the control group (P1<0.05).
- The negative and positive anti-HCV patients groups showed significantly higher ALP level than that in the control group (P1<0.05).
- The positive anti-HCV patients group showed significantly higher total bilirubin group than that in the control group (P1<0.05).

Table VIb: Statistical significance in the serum enzymes and bilirubin between the controls and SHF patients groups

Group / Variable	Controls	SHF Patients	
		-ve anti HCV Abs	+ve anti HCV Abs
AST (Units/L)			
Mean	21.6	30.17	57.6
±S.D.	6.54	15.29	30.58
P1		N. S.	<0.05
P2			<0.05
F: <0.001			
ALT (Units/L)			
Mean	22.5	36.89	49.4
±S.D.	11.1	32.17	41.89
P1		N. S.	<0.05
P2			N. S.
F: <0.05			
ALP (Units/L)			
Mean	132.45	236.88	228.35
±S.D.	36.47	122.37	91.29
P1		<0.05	<0.05
P2			N. S.
F: <0.001			
T. Bil. (mg/dl)			
Mean	0.73	1.1	1.39
±S.D.	0.19	0.55	0.83
P1		N. S.	<0.05
P2			N. S.
F: <0.05			
D. Bil. (mg/dl)			
Mean		0.47	0.6
±S.D.		0.27	0.49
P2			N.S.

SHF = Schistosomal hepatic fibrosis
S.D.= Standard deviation
AST = Aspartate aminotransferase
D. Bil. = Direct bilirubin
ALT = Alanine aminotransferase
ALP = Alkaline phosphatase
T. Bil. = Total bilirubin
P1 : Statistical significance between controls and each of the patient groups.
P2 : Statistical significance between both patients groups.
F: statistical significance between the three studied groups.

Table VII: Cholesterol, HDL-C, LDL-C and triglycerides in controls (mg/dL)

The table shows the results of cholesterol, HDL-C, LDL-C and triglycerides in the control group.

- The total cholesterol ranged from 140-207 mg/dL with a mean value 181.8±19.54 mg/dL.
- The HDL-cholesterol ranged from 35-87 mg/dL with a mean value 48.6±13.21 mg/dL.
- The LDL-cholesterol ranged from 82-141 mg/dL with a mean value 117.55±17.41 mg/dL.
- The triglycerides ranged from 48-160 mg/dL with a mean value 77.35±27.53 mg/dL.

Table VII: Cholesterol, HDL-C, LDL-C and triglycerides in controls (mg/dL)

No.	Total Cholesterol	HDL-Cholesterol	LDL-Cholesterol	Triglycerides
1	158	46	95	85
2	180	38	128	68
3	172	43	119	51
4	189	40	139	54
5	188	46	128	70
6	190	43	115	160
7	206	58	133	74
8	140	44	82	68
9	188	35	139	71
10	201	87	104	49
11	163	48	103	48
12	171	40	110	92
13	151	40	100	58
14	195	60	121	69
15	207	56	141	53
16	156	40	99	85
17	189	43	126	100
18	196	36	141	95
19	201	74	102	124
20	195	55	126	73
Mean	181.8	48.6	117.55	77.35
±S.D.	19.54	13.21	17.41	27.53

HDL-Cholesterol = High Density Lipoprotein-Cholesterol
LDL-Cholesterol = Low Density lipoprotein-Cholesterol

S.D. = Standard deviation

Table VIIIa: Cholesterol, HDL-C, LDL-C and triglycerides in SHF patients with negative anti-HCV antibodies

The table shows the results of cholesterol, HDL-, LDL- and triglycerides in the SHF patients group with negative anti-HCV antibodies.

- The total cholesterol ranged from 109-243 mg/dL with a mean value 166.39±41.11mg/dL.
- The HDL-cholesterol ranged from 18-65 mg/dL with a mean value 38.39±12.82 mg/dL.
- The LDL-cholesterol ranged from 58-176 mg/dL with a mean value 107.61±34.64 mg/dL.
- The triglycerides ranged from 25-160 mg/dL with a mean value 91.76±43.51 mg/dL.

VIIIa: Cholesterol, HDL-C, LDL-C and triglycerides in SHF patients with negative anti-HCV antibodies (mg/dl)

No.	Total Cholesterol	HDL-Cholesterol	LDL-Cholesterol	Triglycerides
1	215	55	130	---
2	139	44	75	103
3	140	19	92	137
4	202	25	127	144
5	152	31	101	101
6	156	34	112	49
7	243	65	157	103
8	240	32	176	160
9	131	30	80	93
10	190	40	138	60
11	182	35	117	152
12	109	37	58	72
13	186	18	139	143
14	111	38	59	71
15	184	44	134	29
16	158	56	97	25
17	129	34	82	64
18	128	54	63	54
Mean ±S.D.	166.39 41.11	38.39 12.82	107.61 34.64	91.76 43.5

SHF = Schistosomal hepatic fibrosis

HDL-Cholesterol = High Density Lipoprotein-Cholesterol
LDL-Cholesterol = Low Density lipoprotein-Cholesterol

S.D. = Standard Deviation

Table VIIIb: Cholesterol, HDL-C, LDL-C and triglycerides in SHF patients with positive anti-HCV antibodies

The table shows the results of cholesterol, HDL-C, LDL-C and triglycerides in the patients group with positive anti-HCV antibodies.

- The total cholesterol ranged from 45-211 mg/dL with a mean value 124.35±44.26 mg/dL.
- The HDL-cholesterol ranged from 10-59 mg/dL with a mean value 33.9±12.85 mg/dL.
- The LDL-cholesterol ranged from 21-135 mg/dL with a mean value 73.75±28.89 mg/dL.
- The triglycerides ranged from 25-153 mg/dL with a mean value 81.15±33.19 mg/dL.

Table VIIIb: Cholesterol, HDL-C, LDL-C and triglycerides in SHF patients with positive anti-HCV antibodies (mg/dL)

No.	Total Cholesterol	HDL-Cholesterol	LDL-Cholesterol	Triglycerides
1	127	38	67	112
2	138	45	83	46
3	122	40	60	110
4	210	55	135	98
5	45	11	24	45
6	60	22	21	39
7	149	36	88	125
8	130	33	87	53
9	137	44	79	74
10	107	25	67	75
11	124	35	70	94
12	92	22	52	72
13	136	25	90	103
14	112	36	63	64
15	160	39	100	107
16	211	59	122	153
17	109	30	66	63
18	92	27	54	56
19	56	10	44	25
20	170	46	103	109
Mean ±S.D.	124.35 44.26	33.9 12.85	73.75 28.89	81.15 33.19

SHF = Schistosomal hepatic fibrosis

HDL-Cholesterol = High Density Lipoprotein-Cholesterol
LDL-Cholesterol = Low Density lipoprotein-Cholesterol

S.D. = Standard deviation

Table IXa: Statistical significance in the studied lipid pattern between the control group and whole SHF patients group

The table shows the statistical significance in the lipid pattern between the control group and whole patients group.

- The patients group showed significantly lower total cholesterol than its level in the controls group ($p<0.001$).
- The patients group showed significantly lower HDL-cholesterol than its level in the controls group ($p<0.001$).
- The patients group showed significantly lower LDL-cholesterol than its level in the controls group ($p<0.001$).

Table IXa: Statistical significance in the lipid pattern between the control group and whole SHF patients group

Group / Variable	Controls	SHF Patients
Number	20	38
Total Chol. (mg/dl)		
Mean	181.8	144.26
±S.D.	19.54	47.28
p		<0.001
HDL-Chol. (mg/dl)		
Mean	48.6	36.03
±S.D.	13.21	12.86
p		<0.001
LDL-Chol. (mg/dl)		
Mean	117.55	89.79
±S.D.	17.41	35.69
p		<0.001
TG (mg/dl)		
Mean	77.35	86.03
±S.D.	27.53	38.1
p		N.S.

SHF = Schistosomal hepatic fibrosis

HDL-Cholesterol = High Density Lipoprotein-Cholesterol
LDL-Cholesterol = Low Density lipoprotein-Cholesterol

S.D. = Standard deviation
p= Statistical significance between the patients and controls groups.

Table IXb. Statistical significance in the studied lipid pattern between the three studied groups

The table shows the statistical differences in the lipid pattern between the three studied groups.

- The SHF patients group with positive anti-HCV showed significantly lower total cholesterol than its level in both the controls and the patients groups with negative anti-HCV (P1, 2<0.05).
- Both SHF patients groups with negative and positive anti-HCV showed significantly lower HDL-cholesterol than the control group (P1<0.05).
- The SHF patients group with positive anti-HCV showed significantly lower LDL-cholesterol than both the controls and the SHF patients groups with negative anti-HCV (P1, 2<0.05).

Table IXb. Statistical significance in the studied lipid pattern between the three studied groups

Group / Variable	Controls	SHF Patients	
		-ve anti HCV Abs	+ve anti HCV Abs
Total Chol. (mg/dl)			
Mean	181.8	166.39	124.35
±S.D.	19.54	41.11	44.26
P1		N. S.	<0.05
P2			<0.05
F: <0.001			
HDL-Chol. (mg/dl)			
Mean	48.6	38.39	33.9
±S.D.	13.21	12.82	12.85
P1		<0.05	<0.05
P2			N. S.
F: <0.05			
LDL-Chol. (mg/dl)			
Mean	117.55	107.61	73.75
±S.D.	17.41	34.64	28.89
P1		N. S.	<0.05
P2			<0.05
F: <0.001			
TG (mg/dl)			
Mean	77.35	91.76	81.15
±S.D.	27.53	43.51	33.19
P1		N. S.	N. S.
P2			N. S.
F: N.S.			

SHF = Shistosomal hepatic fibrosis

HDL-Chol. = High Density Lipoprotein-Cholesterol
LDL-Chol.= Low Density lipoprotein-Cholesterol
TG = Triglycerides

S.D.= Standard deviation

P1 = Statistical significance between controls and each of the patient groups.
P2 = Statistical significance between both patients groups.
F = Statistical significance among the three studied groups

Table Xa: Serum PCIIIP in the studied groups

The table shows the serum PCIIIP levels in the 3 studies groups.

- The control group showed a mean 4.13±1.25 µg/L.
- The patients group with negative anti-HCV showed a mean 4.45±2.22 µg/L.
- The patients group with positive anti-HCV showed a mean 5.88±2.61 µg/L.
- The patients group with positive anti-HCV showed significantly higher PCIIIP than that in both the control and the patients group with negative anti-HCV (P1, 2<0.05).

Table Xa: Serum PCIIIP in the studied groups (µg/L)

Group / No.	Controls	SHF Patients	
		-ve anti-HCV Ab	+ve anti-HCV Ab
1	3.8	2.1	4.9
2	3.3	5.5	5
3	4.5	7.6	7.8
4	6.5	5	3.5
5	5.7	4.5	2.2
6	3	2.6	10
7	2.9	3.6	5
8	2.9	3	8.3
9	4.5	2.9	7.2
10	1.6	10	2.7
11	5	4.7	6
12	3.5	4.9	4.9
13	4.6	2.3	11.5
14	3.8	8.4	3.2
15	3.5	2.5	3
16	6	4.1	3.5
17	4	2.7	8.8
18	4.3	4	5.3
19	6		6.3
20	3.2		8.4
Mean	4.13	4.45	5.88
±S.D.	1.25	2.22	2.61
P1		N. S.	<0.05
P2			<0.05
F: <0.05			

SHF = Schistosomal hepatic fibrosis

S.D. = Standard deviation

P1 = Statistical significance between controls and each of the patient groups.
P2 = Statistical significance between both patients groups.
F = Statistical significance among the three studied groups.

Table Xb: Serum PCIIIP level in controls group and whole SHF patients group

The table shows the statistical significance in the PCIIIP level between the control group and the whole patients group.

The whole patients group showed significantly higher PCIIIP than the controls group ($p<0.05$)

Table Xb: Serum PCIIIP level in controls group and whole SHF patients group (µg/L)

	Controls	Patients
Number	20	38
Mean	4.13	5.21
±S.D.	1.25	2.5
P		<0.05

SHF = Schistosomal hepatic fibrosis

S.D. : Standard deviation

P : Statistical significance between control group and whole patients group.

Table XIa: Serum TGF-β1 in the studied groups

The table shows the values of serum TGF-β1 in the 3 studied groups.

- The control group showed a mean 8.19±1.61 ng/ml.
- The SHF patients group with negative anti-HCV showed a mean 10.49±4.04 ng/ml.
- The SHF patients group with positive anti-HCV showed a mean 17.85±11.86 ng/ml.
- The SHF patients group with positive anti-HCV showed significantly higher TGF-β1 than that in both the control and the SHF patients group with negative anti-HCV (P1, 2<0.05).

Table XIa: Serum TGF-β1 in the studied groups (ng/mL)

Group / No.	Controls	SHF Patients	
		-ve anti-HCV Ab	+ve anti-HCV Ab
1	8.1	11.5	16.9
2	8.6	9.9	16.1
3	8.5	8	10.7
4	7.9	6.5	17.8
5	7.6	7.5	17.3
6	7.5	8.5	27.4
7	10.9	9.6	10.4
8	8	16.9	16.8
9	10.3	7.5	14
10	13.2	8.2	22.9
11	7.5	11.1	16.6
12	7.6	21.3	19.9
13	7.5	8.7	9.8
14	6.8	10	60.4
15	8	7.7	6.4
16	8.2	10	10.8
17	6.5	8.3	13.1
18	7.6	17.7	31.4
19	6.5		10.9
20	6.9		7.3
Mean	8.19	10.49	17.85
±S.D.	1.61	4.043	11.86
P1		N. S.	<0.05
P2			<0.05
F: <0.001			

SHF = Schistosomal hepatic fibrosis

S.D. = Standard deviation

P1 = Statistical significance between controls and each of the patient groups.
P2 = Statistical significance between both patients groups.
F = Statistical significance among the three studied groups

Table XIb: Serum TGF-β1 in the control group and whole SHF patients group

The table shows the statistical significance in the TGF-β1 level between the control group and the whole SHF patients group.

The whole SHF patients group showed significantly higher TGF-β1 than the control group ($p<0.001$).

Table XIb: Serum TGF-β1 in the control group and whole SHF patient group (ng/mL)

	Controls	SHF Patients
Number	20	38
Mean	8.19	14.36
±S.D.	1.61	9.67
p		<0.001

SHF = Schistosomal hepatic fibrosis

S.D. : Standard Deviation

p : Statistical significance between control group and whole patients group.

Table XII: Mean values of TGF-β1 in relation to the Body Mass Index in the studied groups

No significant difference in the serum TGF-β1 level was found between subjects with ideal weight and those obese in any of the studied groups.

Table XII: Mean values of TGF-β1 in relation to the Body Mass Index in the studied groups

Group / Item	Controls		SHF patients			
			-ve anti-HCV Ab		+ve anti-HCV Ab	
	Ideal	Obese	Ideal	Obese	Ideal	Obese
n.	17	3	11	7	14	6
Mean	8.29	7.57	9.55	11.99	17.74	18.1
±S.D.	1.71	0.651	2.97	5.24	14.01	4.85
P		N. S.		N. S.		N. S.

SHF = Schistosomal hepatic fibrosis

n. = Number of patients.
S.D. = Standard deviation

Ideal : ≤25 Kg/m^2
Obese : >25 Kg/m^2

P = statistical significance of TGF-β1 between subjects with ideal weight and obese

Table XIII: Mean values of TGF-β1 and ALT according to the cutoff value for PCIIIP in the SHF patients groups

A cutoff value for PCIIIP was calculated from the mean PCIIIP of the control group + 1 S.D. This gave a value of 5.38 μg/L.

Subjects in each of the patients groups were divided according to this cutoff value into:
Those with normal PCIIIP level (≤5.38 μg/L).

Those with high PCIIIP level: (>5.38 μg/L).

The table shows the mean values of TGF-β1 and ALT according to the cutoff value for PCIIIP in the patients groups.

The TGF-β1 level in the positive anti-HCV patients group with normal PCIIIP level showed significantly higher values than its level in the negative anti-HCV patients group with normal PCIIIP level ($P1<0.05$).

The ALT level and the TGF-β1 level in the positive anti-HCV patients group with high PCIIIP level showed significantly higher values than its level in the negative anti-HCV patients group with high PCIIIP level ($P2<0.05$).

Table XIII: Mean values of TGF-β1 and ALT according to the cutoff value for PCIIIP in the SHF patients groups

Group / No.	SHF Patients			
	-ve anti-HCV Ab		+ve anti-HCV Ab	
PCIIIP level	Normal	High	Normal	High
Number of cases	14	4	11	9
TGF-β1 (ng/mL)				
Mean	10.91	9.03	20.94	14.07
±S.D.	4.5	1.07	14.7	5.89
P		N. S.		N. S.
P1			<0.05	
P2				<0.05
ALT (U/L)				
Mean	39.71	27.0	37.9	63.44
±S.D.	35.66	13.83	35.78	46.5
P		N. S.		N. S.
P1			N. S.	
P2				<0.05

SHF = Schistosomal hepatic fibrosis

S.D. = Standard deviation

P = Significance between patients with normal and high PCIIIP in each of patients group
P1 = Significance between those with normal PCIIIP in both anti HCV –ve and anti HCV +ve
P2 = Significance between those with high PCIIIP in both anti HCV –ve and anti HCV +ve

Table XIVa: The levels of TGF-β1 and PCIIIP in SHF patients group with negative anti-HCV in relation to the high (risky) levels of lipid profile

Cutoff values of the lipid profile were calculated from the mean values of the controls as follows:
Total cholesterol: mean + 1 S.D.
LDL-cholesterol: mean + 1S.D.
Triglycerides: mean + 1S.D.
HDL-cholesterol: mean – 1S.D.

Subjects were divided according to the cutoff values into:
Those with high (risky) levels and those with normal lipid profile (low risk).
The high (risky) levels of the studied lipid profile were:
-Total cholesterol >201.34 mg/dl
-LDL-cholesterol >134.96 mg/dl
-Triglycerides >104.88 mg/dl
-HDL-cholesterol <35.39 mg/dl

There was no statistical significance neither in TGF-β1 nor in PCIIIP levels in patients group with negative anti-HCV in any of the studied lipid pattern.

Table XIVa: The levels of TGF-β1 (ng/ml) and PCIIIP(μg/L) in SHF patients group with negative anti-HCV in relation to the high (risky) levels of lipid profile

	Total Cholesterol		LDL-Cholesterol		HDL-Cholesterol		Triglycerides	
Level	**Normal**	**High**	**Normal**	**High**	**Low**	**Normal**	**Normal**	**High**
number	14	4	14	4	9	9	12	5
% of total	77.8	22.2	77.8	22.2	50	50	70.6	29.4
TGF-β1:								
Mean	10.31	11.13	10.39	10.85	11.77	9.22	10.52	10.45
±S.D.	4.10	4.37	4.18	4.08	4.61	3.14	4.37	3.68
p		N.S.		N.S.		N.S.		N.S.
PCIIIP:								
Mean	4.76	3.43	4.39	4.73	5.01	3.92	4.64	4.12
±S.D.	2.39	1.22	1.88	3.56	2.63	1.71	2.36	2.09
p		N.S.		N.S.		N.S.		N.S.

SHF = Schistosomal hepatic fibrosis

S.D. : Standard Deviation

p : statistical significance between those with low risk of each item and those above that level

Table XIVb: The levels of TGF-β1 and PCIIIP in SHF patients group with positive anti-HCV in relation to the high (risky) levels of lipid profile

Cutoff values of the lipid profile were calculated from the mean values of the controls as follows:
Total cholesterol: mean + 1 S.D.
LDL-cholesterol: mean + 1S.D.
Triglycerides: mean + 1S.D.
HDL-cholesterol: mean – 1S.D.

Subjects were divided according to the cutoff values into:
Those with high (risky) levels and those with normal lipid profile.
The high (risky) levels of the studied lipid profile were:
-Total cholesterol >201.34 mg/dl
-LDL-cholesterol >134.96 mg/dl
-Triglycerides >104.88 mg/dl
-HDL-cholesterol <35.39 mg/dl

There was no statistical significance in neither TGF-β1 level nor in PCIIIP level in patients group with positive anti-HCV in any of the studied lipid pattern.

Table XIVb: The level of TGF-β1 (ng/ml) and PCIIIP (μg/L) in SHF patients group with positive anti-HCV in relation to the high (risky) levels of lipid profile

	Total Cholesterol		LDL-Cholesterol		HDL-Cholesterol		Triglycerides	
Level	**Normal**	**High**	**Normal**	**High**	**Low**	**Normal**	**Normal**	**High**
number	18	2	19	1	10	10	14	6
% of total	90	10	95	5	50	50	70	30
TGF-β1:								
Mean	18.24	14.3	17.85	17.8	17.08	18.61	21.03	10.42
±S.D.	12.41	4.95	12.18	--	15.71	6.97	12.8	3.69
p		N.S.		N.S.		N.S.		N.S.
PCIIIP:								
Mean	6.14	3.5	6.0	3.5	5.15	6.6	6.06	5.43
±S.D.	2.62	0.0	2.62	--	2.0	13.04	2.71	2.2
p		N.S.		N.S.		N.S.		N.S.

SHF : Schistosomal hepatic fibrosis
S.D. : Standard Deviation

p : statistical significance between those with low risk of each item and those above that level.

Table XIVc: The level of TGF-β1 in both SHF patients subgroups in relation to the low (unrisky) levels of lipid profile

Cutoff values of the low level of lipid profile were calculated from the mean values of the controls as follows:
Total cholesterol: mean - 1 S.D.
LDL-cholesterol: mean - 1S.D.

Subjects were divided according to the cutoff values into:
Those with low risk levels and those with normal lipid profile.
The low risk levels of the studied lipid profile were:
-Total cholesterol <162.26 mg/dl
-LDL-cholesterol <100.14 mg/dl

Table XIVc: The level of TGF-β1 (ng/ml) in both SHF patients subgroups in relation to the low (unrisky) levels of lipid profile

	Pure Schistosomiasis subgroup				Mixed infection subgroup			
	Cholesterol		LDL-cholesterol		Cholesterol		LDL-cholesterol	
	Low	Normal	Low	Normal	Low	Normal	Low	Normal
Number	10	4	8	6	17	1	16	3
%	55.6	22.2	44.4	33.3	85	5	80	15
TGF-β1								
Mean	10.87	8.93	11.59	8.8	18.88	7.3	19.66	8.17
±S.D.	4.73	1.51	5.07	2.04	12.48	--	12.45	2.33
p		N.S.		N.S.		N.S.		<0.05

SHF = Schistosomal hepatic fibrosis

S.D.: Standard deviation

p: statistical significance between cases with normal and low lipid levels in each of the patients subgroups.

Table XVa: PCIIIP and TGF-β1 levels according to the grade of periportal fibrosis in SHF patients group with pure schistosomiasis (-ve anti-HCV)

Only the PCIIIP level in patients with grade III periportal fibrosis (PPF), showed significantly higher levels than that in patients with grade I PPF. The TGF-β1 level was higher with increasing grades of PPF, though not reaching a significant level.

Table XVa: PCIIIP (µg/L) and TGF-β1 (ng/ml) levels according to the grade of periportal fibrosis in SHF patients group with pure schistosomiasis (-ve anti-HCV)

Grade / Serial no.	I			II			III		
	Pt.no.	PCIIIP	TGF-β1	Pt.no.	PCIIIP	TGF-β1	Pt.no.	PCIIIP	TGF-β1
1	1	2.1	11.5	3	7.6	8.0	2	5.5	9.9
2	6	2.6	8.5	4	5.0	6.5	10	10.0	8.2
3	7	3.6	9.6	5	4.5	7.5	11	4.7	11.1
4	9	2.9	7.5	8	3.0	16.9	14	8.4	10.0
5	13	2.3	8.7	12	4.9	21.3	18	4.0	17.7
6	17	2.7	8.3	15	2.5	7.7			
7				16	4.1	10.0			
Number		6	6		7	7		5	5
Mean		2.7	9.02		4.51	11.13		6.52	11.38
±S.D.		0.53	1.39		1.65	5.69		2.52	3.68
P					N.S.	N.S.			
P1								<0.005	N.S.
P2								N.S.	N.S.
F:	For PCIIIP: <0.05 and for TGF-β1: N.S.								

SHF = Schistosomal hepatic fibrosis

Pt. no. Patient number

S.D. : Standard Deviation
P : Statistical significance between patients with grade I and II periportal fibrosis.
P1 : Statistical significance between patients with grade I and III periportal fibrosis
P2 : Statistical significance between patients with grade II and III periportal fibrosis
F : Statistical significance among the studied groups

Table XVb:PCIIIP and TGF-β1 levels according to the grade of periportal fibrosis in SHF patients group with mixed fibrosis and cirrhosis (+ve anti-HCV)

Only the PCIIIP in patients with grade III PPF showed significantly higher levels than its level in patients with both grade I and II PPF.

The TGF-β1 level was higher in patients with grade II than that in grade I PPF, though not reaching a significant level.

Table XVb: PCIIIP (µg/L) and TGF-β1(ng/ml) levels according to the grade of periportal fibrosis in SHF patients group with mixed fibrosis and cirrhosis (+ve anti-HCV)

Grade / Serial	I			II			III		
	Pt.no.	PCIIIP	TGF-β1	Pt.no.	PCIIIP	TGF-β1	Pt.no.	PCIIIP	TGF-β1
1	1	4.9	16.9	3	7.8	10.7	6	10.0	27.4
2	2	5.0	16.1	4	3.5	17.8	7	5.0	10.4
3	9	7.2	14.0	5	2.2	17.3	13	11.5	9.8
4	10	2.7	22.9	8	8.3	16.8	17	8.8	13.1
5	11	6.0	16.6	14	3.2	60.4	18	5.3	31.4
6	12	4.9	19.9				20	8.4	7.3
7	15	3.0	6.4						
8	16	3.5	10.8						
9	19	6.3	10.9						
Number		9	9		5	5		6	6
Mean		4.83	14.94		5.00	24.60		8.17	16.57
±S.D.		1.53	5.04		2.83	20.22		2.58	10.19
P					N.S.	N.S.			
P1								<0.05	N.S.
P2								<0.05	N.S.
F:	For PCIIIP : <0.05 and for TGF-β1: N.S.								

SHF : Schistosomal hepatic fibrosis

Pt. no: Patient number

S.D. : Standard Deviation
P : Statistical significance between SHF patients with grade I and II periportal fibrosis.
P1 : Statistical significance between SHF patients with grade I and III periportal fibrosis
P2 : Statistical significance between SHF patients with grade II and III periportal fibrosis
F : Statistical significance among the studied groups

Table XVI:Prevalence of cases having TGF β1 and PCIIIP levels above the cutoff levels (mean of the levels in the controls + 1 S.D.)

The prevalence of cases with high PCIIIP and TGF-β1 was more in the group of patients with positive anti-HCV. This is because:

- In the SHF patients group with negative anti-HCV, 4 cases out of 18 (22.2%) had higher PCIIIP and 8 cases (44.4%) had high TGF-β1 levels.
- In the SHF patients group with positive anti-HCV, 9 out of 20 cases (45%) had higher PCIIIP and 17 cases (85%) had higher TGF-β1 levels.

Table XVI:Prevalence of cases having TGF-β1 and PCIIIP levels above the cutoff levels (mean of the levels in the controls + 1 S.D.)

	SHF patients			
	-ve anti-HCV		+ve anti-HCV	
	High PCIIIP	High TGF-β1	High PCIIIP	High TGF-β1
Number of case	4	8	9	17
% Prevalence	22.2	44.4	45	85

SHF: Schistosomal hepatic fibrosis

S.D. : Standard deviation.

Table XVII:Significant correlations

1. Significant Correlations in the control group (n=20)

Item	with: Item	r	p
TGF-β1	PCIIIP	-0.4671	<0.05
PCIIIP	TGF-β1		
	Fasting blood glucose	-0.4555	<0.05
AST	ALT	0.6138	<0.05
ALT	AST		
	LDL-Cholesterol	-0.4471	<0.05
Total Cholesterol	HDL-Cholesterol	0.4824	<0.05
	LDL-Cholesterol	0.7198	<0.001

2. Significant correlations in the SHF patients group with negative anti-HCV (n=18)

Item	with: Item	r	p
TGF-β1	Total bilirubin	-0.4749	<0.05
AST	ALP	0.6879	<0.05
	ALT	0.4952	<0.05
Total bilirubin	Direct bilirubin	0.6164	<0.05
Total cholesterol	LDL-Cholesterol	0.9570	<0.001
HDL-Cholesterol	Triglycerides	-0.5519	<0.05

3. Significant correlations in SHF patients group with positive anti-HCV (n=20)

Item	with: Item	r	p
PCIIIP	ALP	0.5613	<0.05
	AST	0.5823	<0.05
AST	PCIIIP		
	ALT	0.7373	<0.001
Total bilirubin	HDL-Cholesterol	-0.5336	<0.05
	Direct bilirubin	0.9033	<0.001
Total Cholesterol	HDL-Cholesterol	0.9204	<0.001
	LDL-Cholesterol	0.9718	<0.001
	Triglycerides	0.7739	<0.001
LDL-Cholesterol	Total Cholesterol		
	HDL-Cholesterol	0.8287	<0.001
	Triglycerides	0.6772	<0.05
HDL-Cholesterol	Total Cholesterol		
	Total bilirubin		
	LDL-Cholesterol		
	Triglycerides	0.6723	<0.05

DISCUSSION

DISCUSSION

Growth factors are polypeptides produced by cells that act to stimulate or inhibit proliferation of either the same cells or other cells. [1]

Transforming growth factor-beta (TGF-β) is a member of a large family of growth factors, which are synthesized by a wide range of cells, where platelets and activated macrophages are the richest sources.[16,186-188] TGF-β can also be derived from the stimulated (injured) endothelial cells.[186,189]

Three main isoforms of TGF-β are recognized in humans, which are structurally similar in the c-terminal region. They are designated TGF-β1, 2 and 3. All have the same functions in respect to their regulation of cellular growth and proliferation.[190] After being formed as secretory precursor polypeptide molecules (latent forms), they require proteolytic cleavage for activation to form a 25 kDa homodimeric peptide.[7,9] The activators of the latent TGF-β are site and function dependent.[191,192]

Transforming growth factor-beta increases the production of many extracellular matrix (ECM) proteins by fibroblasts or hepatic Ito and Kupffer cells, such as fibronectin, collagen types I, III, VI, tenascin, elastin, osteonectin, thrombospondin, byglycan and decorin.[5,94,95] It also downregulates the production of collagenase, stromelysin, elastase, plasminogen activator and other proteinases involved in ECM destruction.[100]

An increase in TGF-β1 has been found in correlation with increased production of collagen mRNA and increased PCIIIP serum levels. [89,90] This might suggest that TGF-β1 has a fibropathogenic role. [193]

It was reported that TGF-β1 was implicated in the pathogenesis of pulmonary and hepatic fibrosis.[194,195] Hepatic schistosomiasis is the most prevalent form of hepatic fibrosis.[139] The final sequelae to schisotome eggs trapped in the liver is periportal fibrosis (PPF) with deposits of collagen in expanded portal tracts,[196] in addition to the formation of intense granulomatous inflammatory response.[139]

The steady progression of liver fibrosis is at least, in part, responsible for the anatomical hemodynamic and functional disturbances leading to portal hypertension and oesophageal variceal bleeding.[197,198] The net increase in liver connective tissues may be caused by their increased biosynthesis, decreased degradation or a combination of both.[199]

Therefore, it was worthy to study in this work the fibropathogenic role of TGF-β1 in patients with hepatic schistosomiasis.

This was done in 58 subjects including 38 patients with schistosomal hepatic fibrosis (SHF) and 20 normal healthy volunteers of comparable age, sex and socioeconomic state as patients. All patients were normotensive and with normal fasting blood glucose level.

The TGF-β1 in the control group had a mean value of 8.19±1.61 ng/ml. (Table XIa).

Other workers reported higher values of about 18 ng/ml, [200,201] and another study reported lower values of about 3 ng/ml.[202] These alterations could be due to racial, methodological or age variations.

In all the studied patients with schistosomal hepatic fibrosis (SHF), the TGF-β1 had a mean value of 14.36±9.67 ng/ml, which was significantly higher than the control group ($p<0.001$) (Table XIb).

No other data about TGF-β1 in patients with SHF are so far available. However, it was reported that in cases with cirrhosis, such as alcoholic cirrhosis,[203] autoimmune hepatic fibrosis[204] and hepatitis C virus infection,[202] the TGF-β production was increased.[91,205]

An increase in TGF-β1 has been found in correlation with the increased histological activity index of fibrogenesis, i.e. collagen mRNA and with increased serum PCIIIP levels.[89,90]

Procollagen III peptide is known to be an index of active fibrogenesis[206-208] and a marker of ongoing fibrosis.[206-210] It was considered superior to other available markers of fibrosis, in terms of sensitivity and predictive value.[210,211] Many studies reported high levels of PCIIIP in patients with SHF.[210,212-216]

In the present study, the PCIIIP level in the control group showed a mean of 4.13±1.25 µg/L (Table Xa). While its level in the whole patients group with SHF was 5.21±2.5 µg/L, showing a significantly higher values than that in the control group (Table Xb). Zaki et al, [216,217] reported an increase in PCIIIP in patients with SHF. They correlated the increase in its level with the degree of hepatocellular integrity as monitored by aminotransferases activity.

In the present study, the activities of aminotransferases and alkaline phosphatase enzymes in the whole SHF patients group were significantly higher than their corresponding activities in the control group (Table VIa).

The degree of fibrosis was also evaluated in this study by ultrasonography (U/S) on the liver, and correlation of PCIIIP with the degree of fibrosis was done (Table XVa,b). The highest values of PCIIIP were observed in cases with marked fibrosis (Grade III). This agrees with previous studies, which reported that the level of PCIIIP in schistosomal patients was more increased as the liver fibrosis became more advanced.[213,217]

Ohmae et al,[212] found that PCIIIP strongly correlated with the development of hepatic fibrosis and portal hypertension. They concluded that the U/S liver patterns along with the increase of serum levels in PCIIIP provided useful indicators for monitoring schisotosoma japonicum infection. However, Kardoff et al,[215] found that PCIIIP is not useful as a diagnostic serum marker of fibrosis in hepatosplenic schistosomiasis.

In addition, Sharon et al[190], reported that although fibrotic manifestations appear to be dependent on TGF-β1 levels, fibrosis is not eliminated in the absence of TGF-β1, nor it is overwhelmed in over-expression of this fibrogenic peptide.

In the present study, the level of the TGF-β1 was also evaluated in mixed schistosomal and hepatitis C virus infection. So, the patients group was subdivided into two subgroups, eighteen patients with pure SHF and 20 had superadded hepatitis C virus infection. All were negative for HBs-Ag.

In the present study, there was no significant difference in the TGF-β1 level in relation to the degree of fibrosis in either patients with pure schistosomiasis or with mixed infection (Table XVa,b). In addition, there

was no significant correlation between TGF-β1 and PCIIIP in either of the patients groups (Table XVII).

In the patients group with pure schistosomiasis (negative anti-HCV antibodies), the TGF-β1 had a mean value of 10.49±4.043 ng/ml (table XIa), which was apparently higher than the value in the control group, but did not reach the level of significance. Eight cases of this pure schistosomal patients (44.4%) had higher TGF-β1 values (Table XVI) than the cutoff value [mean of its level in controls + 1SD = 9.8 ng/ml].

In this pure schistosomal subgroup, the PCIIIP level, which is the monitor for active fibrosis, showed a mean value of 4.45±2.22 μg/L (Table Xa). This level showed no significant difference from that in the control group. Four patients of them (22.2%) had higher PCIIIP values (Table XVI) than the cutoff value [mean of its level in controls + 1SD = 5.38 μg/L]. In addition, there was no significant correlation between TGF-β1 and PCIIIP in these pure schistosomal patients (Table XVII).

The lack of significant differences in TGF-β1 and PCIIIP levels between the pure schistosomiasis group and the control group (Table Xa and XIa) can be explained by the presence of mild degree of PPF. Eleven cases out of 18 (61.1%) had normal portal vein (Table IIa), as monitored by U/S, adding more evidences of the presence of a mild degree of PPF.

Hepatic fibrosis was reported to be the healing response to hepatic injury.[218] It has been suggested that after hepatic injury, when tissue repair is complete, an unknown mechanism shuts down the TGF-β expression and the ensuing matrix production.[88] Although schistosomal hepatic infection is usually mesenchymal and does not affect the hepatic cells, the integrity of hepatic cell has been reported to be affected early in

the disease [219,220]. Moreover, a study reported that the intracellular deposition of fat within hepatic cells might be due to toxins released by S. mansoni which causes ischaemia of liver parenchyma following the oviposition.[221]

In early liver injury, there is replacement of the normal subendothelial matrix, which contains laminin, type IV collagen and fibronectin, by another matrix enriched in interstitial collagen types I and III.[222]This replacement leads to deterioration of hepatocellular functions and alters the biology of other cells.[120]

In the present study, 5 patients out of 18 (27.8%) with pure schistosomiasis (cases no. 2, 4, 8, 13, 18) had high alanine aminotransferase activity (>40 U/L) (Table Va). This denoted the presence of mild hepatocellular affection in these patients.

It is known that TGF-β is expressed in response to any insult to the hepatic cell.[82,89,90] This could be a second possible explanation for the relative increase in the TGF-β1 observed in the pure schistosomal patients of the present study, in addition to the presence of mild degree of PPF.

It is known that hepatic injury and ongoing fibrosis (cirrhosis) is more marked with chronic viral infection. A superadded hepatitis C viral infection is frequent in hepatic schistosomiasis.[146] It was found that patients with hepatic schistosomiasis have higher rates of hepatitis C virus seropositivity.[145] This association is believed to be, partly, due to transmission of hepatitis virus during blood transfusion and/or parenteral therapy for schistosomiasis with contaminated needles.[148]

Some studies failed to demonstrate a definite association between chronic infection with hepatitis C virus and ultrasonographic or histologic

evidence of SHF.[132,223] In addition, another study reported the possibility of false positive anti-HCV tests secondary to cross reaction with anti-schistosomal antibodies. [224]

In the present work, twenty schistosomal patients who were seropositive for anti-HCV antibodies were also studied. Their hepatic ultrasonographic picture showed that all cases had PPF and coarse echogenic pattern of mixed fibrosis and cirrhosis. This picture was due to the superadded infection with hepatitis C virus on hepatic schistosomiasis.

In these patients, the mean value of the TGF-β1 level was 17.85±11.86 ng/ml (Table XIa), which was significantly higher than that in both the control group and the pure schistosomiasis patients. In addition, the PCIIIP level for this group of mixed infection showed a mean value 5.88±2.61 μg/L, which was also significantly higher than that in both the control group and the patients group with negative anti-HCV antibodies (Table Xa). The significant increase in both PCIIIP and TGF-β1, in patients with superadded HCV infection more than the corresponding levels in patients with pure schistosomiasis denoted the occurrence of more fibrosis and more expression of TGF-β1 in patients with mixed infection.

Seventeen out of these twenty patients (85%) with mixed infection (Table XVI) had high TGF-β1 above cutoff value of 9.8 ng/ml. Nine of these patients (45%) showed high PCIIIP level (above the cutoff value) (Table XVI). Therefore, the prevalence of cases with high TGF-β1 and PCIIIP levels was more in patients with mixed infection than in the patients with pure schistosomiasis (Table XVI).

Flisiak et al,[201] suggested that TGF-β1 measurement would indicate liver function impairment and act as a possible marker of the progression of hepatic fibrosis in cirrhotic patients. It was reported that TGF-β1 level could be a useful marker in assessing the situation of active liver fibrogenesis in patients with chronic viral hepatitis.[90,200,225] It was reported that the severer the hepatocellular injury, the more marked the degree of fibrosis.[226,227]

According to the cutoff value for PCIIIP (5.38 μg/L); patients with mixed infection who had high PCIIIP levels showed significantly higher ALT activity and TGF-β1 level when compared with patients with pure hepatic schistosomiasis who had high PCIIIP level (table XIII). These findings indicated the presence of both hepatocellular injury and an active fibrogenic process.

Aspartate aminotransferase (AST) was reported to be a known monitor of chronic hepatitis and cirrhosis.[217]

In the present study, the patients group with positive anti-HCV antibodies showed higher activity of AST than its level in both controls and patients group with pure schistosomiasis (Table VIb). Eleven of the 20 cases with mixed infection had AST activity more than 40 U/L. In addition, activities of alanine aminotransferase (ALT) and alkaline phosphatase (ALP) as well as total bilirubin level showed higher values than their levels in controls (Table VIb). This agrees with other studies which found increased levels of aminotransferases and bilirubin as well as marked PPF in patients with mixed hepatic schistosomiasis and HCV infection.[144,228]

The presence of significantly higher levels of both TGF-β1 and PCIIIP in the group with mixed infection (Tables Xa and XIa) agrees with the studies done by Murawaki et al[225] and Nelson et al[200]. They suggested that TGF-β1 is an important factor in the process of fibrogenesis through collagen deposition in the ECM.

In the present study, the ultrasonographic picture of the liver of the patients with mixed infection showed that 13 cases (65%) had dilated portal vein (Table IIb), which indicated the presence of more advanced fibrosis.

On the other hand, several studies associated TGF-β1 expression with the development of arterial disease,[229-233] whereas others suggested that TGF-β1 expression prevents the formation of arterial lesion, especially atherosclerosis.[161,234-236] Atherogenicity implies changes in the vessel wall and changes in some plasma constituents such as the lipid profile.[237]

Endothelial activation by oxidized lipoproteins plays an important role in the initiation of atherosclerotic lesion through increased adhesion of mononuclear cells and their recruitment into the vascular wall.[237] The recruited inflammatory cells induce and produce the expression of numerous inflammatory cytokines and chemokines promoting lesion progression.[238,239]

High TGF-β level has been reported to increase the risk for accelerated atherosclerosis in obese subjects.[240]

In the present study, 25 patients out of the 38 (65.8%) had ideal body weight [Body mass index (BMI)≤ 25 Kg/m^2] and only one case (No. 5 in the group with mixed infection) had a BMI 39 Kg/m^2 (Tables IIa,b). On dividing the patients according to their BMI, no significant differences

were found in the TGF-β1 levels between patients with ideal body weight and the obese patients (Table XII). This will nullify the role of obesity on the presence of high TGF-β1 levels in schistosomal patients.

A good number of studies found that serum levels of TGF-β were markedly lower in patients with coronary atherosclerosis than in those without.[61-63,161,234,241-244] However, these results have been challenged by other workers[245-247] who reported that no direct causal relationship between TGF-β activity and atherosclerosis.

This controversy has been attributed to differences in pre-analytic design, measurement in different matrices,[161,245] the use of different methods for measurement of TGF-β and lack of international reference material for proper standardization of the test.[248]

TGF-β can be considered to have a "double-edged sword" effect. It may reduce the atherogenesis by inhibiting smooth muscle cell proliferation. However, when there is ongoing vessel wall injury, the TGF-β1 can be synthesized from the stimulated (injured) endothelial cells[186,189] and atherogenesis may be enhanced by promoting excessive ECM accumulation. The outcome could represent a complex balance between these two competing influences.[245-252]

Transforming growth factor – beta1 inhibits the proliferation and migration of vascular smooth muscle cells, promotes formation and secretion of protease inhibitors, has an anti-inflammatory function, suppresses the macrophages activation and leukocyte adhesion to endothelial cells.[161,188,189,248,253-256] All these properties of TGF-β suggest its potential anti-atherogenic role.[257]

In addition, Mallat et al[258] pointed to an important protective role of endogenous TGF-β in both plaque development and composition. This protective effect seems to depend on the potent deactivating effects of TGF-β on macrophages and T-lymphocytes. But, it does not seem to be related to its effects on smooth muscle cell differentiation and/or accumulation.

Some theories for atherosclerosis suggested that low injury to the arterial wall will lead to inflammatory insudation of cellular and plasma constituents in the intima of the artery.[238,248]

Many risk factors have been associated with atherosclerosis of which the dyslipidaemia is an important contributor.[237,259,260]

Advanced schistosomal hepatic fibrosis was thought to be atheroprotective, since the incidence of atherosclerosis is not common in these patients.[261] This low incidence has been attributed to hyperestrogenemia,[164,168,174] hypocholesterolaemia,[165,169] hypocoagulability and hyperfibrinolysis[176] found in these patients.

In the present study, none of the studied patients showed clinical or electrocardiographic signs of atherosclerosis. The total cholesterol levels in the whole patients had a mean value of 144.26±47.28 mg/dl, the HDL-cholesterol had a mean value of 36.03±12.86 mg/dl, the LDL-cholesterol had a mean value of 89.79±35.69 mg/dl and the triglycerides (TG) had a mean value of 86.03±38.1 mg/dl (Table IXa). Although all these levels were within the normal ranges, the cholesterol levels (Total and HDL- or LDL- fractions) showed significantly lower levels when compared with their levels in controls.

As regarding the lipid profile in the subgroups of SHF patients, the patients with pure schistosomiasis had a mean value for total cholesterol of 166.39±41.11 mg/dl, HDL-cholesterol of 38.39±12.82 mg/dl, LDL-cholesterol of 107.61±34.64 mg/dl and TG of 91.76±43.51 mg/dl (Table IXb); whereas in patients group with positive anti-HCV antibodies, the mean value for total cholesterol was 124.35±44.26 mg/dl, that of HDL-cholesterol was 33.9±12.85 mg/dl, that of LDL-cholesterol was 73.75±28.89 mg/dl and that of TG was 81.15±33.19 mg/dl (Table IXb).

On comparing the lipid profiles in both subgroups of patients with the control values, the only significant finding in the subgroup with pure schistosomal infection was the significant decrease in HDL-cholesterol. The significant decrease in the HDL-cholesterol than that in the controls was due to the overall decrease in the total cholesterol level found in these patients. In the subgroup with mixed infection, both the total cholesterol level and its high- and low- density fractions showed significantly lower levels when compared with the corresponding levels in controls (table IXb).

In addition, on considering the mean control values for lipid profile ± 1 S.D. as cutoff values for the low and high levels, the low (unrisky) levels were calculated as being <162.26 mg/dl for total cholesterol, <100.14 mg/dl for LDL-cholesterol, <49.82 mg/dl for TG and >61.8 mg/dl for HDL-level.

In the subgroup of pure schistosomal infection, 10 cases (55.6%) had low total cholesterol level, and 8 cases (44.4%) had low LDL cholesterol level. While in the subgroup of mixed infection, 17 cases (85%) had low total cholesterol level and 16 cases (80%) had low LDL-cholesterol level (Table XIVc). These results denoted the high prevalence of low (unrisky)

lipid levels, especially in patients with mixed infection. On the other hand, the risky levels were calculated as being >201.34 mg/dl for total cholesterol, >134.96 mg/dl for LDL-cholesterol, >104.88 mg/dl for TG and <35.39 mg/dl for HDL-cholesterol (Tables XIVa,b). Only six cases of the whole patients in both subgroups (15.8%) had risky total cholesterol level (higher than the cutoff value); four of them were in the group of pure schistosomal infection. Five cases from the whole patients (13.2%) had high (risky) LDL-cholesterol level; four of them were in the group of pure schistosomiasis. Eleven from the whole patients (28.9%) had risky TG level; five of them were in the group of pure schistosomiasis infection. All these results denoted the low prevalence of cases with high lipid profiles in schistosomal patients especially those with positive anti-HCV Ab. The presence of both high prevalence of low (unrisky) lipid levels and the low prevalence of high (risky) levels can explain the uncommon occurrence of atherosclerosis in schistosomal patients.

As regards the TGF-β1 levels in patients with low, normal and high lipid profile, no significant differences could be observed between its level in both subgroups of patients in relation to the changes in the lipid profile (Tables XIVa-c), except for its level in schistosomal patients with HCV infection who had low level of LDL-cholesterol. The TGF-β1 level in this subgroup of patients was significantly higher than its level in those with normal LDL-cholesterol level. In addition, the TGF-β1 level in those with low total cholesterol level in both groups of patients (negative HCV and positive HCV) as well as in those with pure schistosomiasis and low LDL-cholesterol level that showed relatively higher levels than their corresponding levels in those with normal cholesterol levels (Table XIVc). The insignificant rise in the TGF-β1 levels could be due to the small sample size of the subgroups. Moreover, no differences were

noticed in the degree of fibrogenesis as evidenced by the PCIIIP levels (Table XIVa and XIVb). Therefore, It can be said that the TGF-β1 levels showed relative rise in relation to the low (unrisky) lipid profile in patients with hepatic schistosomiasis especially when accompanied by HCV infection. This may indicate an atheroprotective effect.

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

Transforming growth factor-beta1 (TGF-β1) is a polypeptide, synthesized by a wide range of cells. It is formed as a secretory precursor (latent form), where proteolytic cleavage is required for its activation to form a 25 kDa homodimeric peptide.

It is not only a potent stimulator of new connective tissue synthesis, but it is also the most potent inhibitor of smooth muscle proliferation. It stimulates the production of extracellular matrix (ECM) proteins and down-regulates the production of collagenase, stromelysin, elastase, plasminogen activator and other proteinases involved in the destruction of ECM. On the other hand, it inhibits the proliferation and migration of vascular smooth muscle cells, has an anti-inflammatory function, suppresses macrophage activation and leukocyte adhesion to endothelial cells and prevents intima formation.

It was reported that, TGF-β1 was implicated in the pathogenesis of hepatic fibrosis. Hepatic schistosomiasis is the most prevalent form of hepatic fibrosis in Egypt. Periportal fibrosis is its final sequel, which is caused by an increase in liver connective tissues due to an increase in their biosynthesis, decrease in their degradation or a combination of both.

Hyperestrogenemia, hypolipidemia, hyperfibrinolysis and hypocoagulability are common findings in schistosomal hepatic fibrosis (SHF) patients. These findings make these patients more atheroprotected.

The aim of the present work was to estimate the serum level of Transforming growth factor-beta 1 in patients with SHF, in an attempt to evaluate its fibropathogenic and atheroprotective roles.

The study was conducted on 58 subjects divided into three groups:

- Group I: It consisted of twenty healthy volunteers (17 males and 3 females) of comparable age, sex and socioeconomic state as patients.
- Group II: It consisted of eighteen patients (13 males and 5 females), diagnosed as pure SHF, who were negative for both hepatitis B surface antigen (HBs Ag) and anti-hepatitis C virus (HCV) antibodies.
- Group III: It consisted of twenty patients (16 males and 4 females), diagnosed as mixed infection of hepatic schistosomiasis and HCV. All were negative for HBs Ag.

All the studied patients were selected free from diseases that can alter the serum TGF-β1 level. They were all normotensive and non-smokers. This selection was done by history taking, clinical examination and/or laboratory means.

All the studied groups were subjected to:

- Full history taking, clinical examination and calculation of the body mass index (BMI). ECG, abdominal ultrasonography and plain X-ray chest were also done.
- Laboratory investigations including serodetection of HBs antigen and anti-HCV antibodies, estimation of the fasting serum glucose, total bilirubin, direct bilirubin, total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides levels, as well as serum activities of AST, ALT, ALP. Serum levels of PCIIIP and TGF-β1 were also estimated.

In the whole patients group, the TGF-β1 and the PCIIIP levels were significantly higher than those in the control group.

The activities of aminotransferases and alkaline phosphatase enzymes in the whole patients group, were significantly higher than their corresponding activities in the control group.

The degree of fibrosis was evaluated by ultrasonography on the liver, the highest levels of serum PCIIIP was found in cases with marked fibrosis. However, no significant difference was found in the serum TGF-β1 in relation to the degree of fibrosis.

In the patients group with pure schistosomiasis, the serum levels of TGF-β1 and the PCIIIP showed a relative increase than those in controls, but did not reach the level of significance. Eight of them (44.4%) had higher TGF-β1 level than the cutoff value (mean of its level in controls + 1 S.D). Also, four of them (22.2%) had higher PCIIIP level above the cutoff value. These results denoted the presence of mild degree of fibrosis and TGF-β1 over-expression. In addition, five patients out of 18 had high alanine aminotransferase level, which denoted the presence of mild hepatocellular affection in these patients.

The hepatic ultrasonographic picture of the subgroup of patients with mixed schistosomal and hepatitis C viral infections showed a pattern of mixed fibrosis and cirrhosis.

In the patients subgroup with positive anti-HCV antibodies and schistosomiasis, both the TGF-β1 and PCIIIP levels were significantly higher than their corresponding levels in both the control group and subgroup of patients with pure schistosomiasis.

The prevalence of cases with high TGF-β1 and PCIIIP was more in patients group with mixed infection, being 85% and 45%; respectively. No significant difference in the TGF-β1 level was found in relation to the

degree of fibrosis. However, the highest values of PCIIIP were observed in cases with marked fibrosis (Grade III). In addition, there was no significant correlation between TGF-β1 and PCIIIP in this patients group.

On comparing patients with high PCIIIP in both subgroups, the TGF-β1 level and the ALT activity were significantly higher in those with mixed infection than in those with pure schistosomiasis. These findings indicated the presence of both hepatocellular injury and active fibrogenic process.

On the other hand, since schistosomal patients were thought to be atheroprotected, the association of TGF-β1 and atherogenesis was also evaluated.

Both the whole patients and their two subgroups had significantly lower cholesterol (Total and LDL-C density fraction) than the control group. On dividing the lipid profiles according to their cutoff values (mean±1 SD of their levels in controls) into low and high levels. There was a high prevalence of the low (unrisky) levels in both subgroups (Table XIVc) especially in those with mixed schistosomal and HCV infection. These results explain the uncommon occurrence of atherosclerosis in the schistosomal patients either alone or mixed with HCV infection.

However, there were no significant differences in the TGF-β1 level in both patients subgroups in relation to high (risky) level of lipid pattern. Also, none of the studied patients showed clinical or electrocardiographic signs of atheroscelerosis. On the other hand, there was a relative rise in TGF-β1 in patients with low levels (unrisky). This relation became

significant in the subgroup with mixed schistosomiasis and HCV infection.

As a conclusion:

◊ TGF-β1 level showed no changes in pure schistosomiasis but its level increased in cases of schistosomal hepatic fibrosis and positive anti-HCV antibodies.

◊ The procollagen III peptide levels were significantly high in cases of SHF accompanied by HCV infection and became higher with the advancement of fibrosis, confirming its relation to the degree of fibrosis.

◊ There was no relation between the level of TGF-β1 and the degree of fibrosis. Therefore, TGF-β1 alone is not a good monitor of fibrosis in cases with pure schistosomal hepatic fibrosis.

◊ Procollagen III peptide is a better monitor for the degree of fibrosis in such cases, being highest in cases with advanced fibrosis.

◊ An increase in TGF-β1 level in SHF patients may point to the presence of a concomitant viral infection specially if accompanied with an increased PCIIIP level.

◊ Anti-atherogenicity in cases of SHF can be attributed to the presence of both hypolipidemia and probably the increase in serum TGF-β1 levels.

Lastly, from all the above results, the hypothesis that TGF-β1 has a role in the formation and progression of fibrosis has been confirmed. On the other hand, its atheroprotective effect, although not clarified, cannot be ruled out.

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PROTOCOL

بسم الله الرحمن الرحيم

Transforming growth Factor-beta1 In Patients With Hepatic Schistosomiasis

معامل التحول النموي – بيتا ١ في مرضى التليف الكبدي البلهاريسي

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إيفاء جزئيا للحصول على

Master Degree

درجة الماجستير

In Chemical Pathology

في الباثولوجيا الكيميائية

By

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Introduction

Transforming growth factor-beta (TGF-β) is a member of a large family of growth factors, which are synthesized by a wide range of cells, where platelets and activated macrophages are the richest sources [1-4]. TGF-β can also be derived from the stimulated (injured) endothelial cells [4,5].

Three isoforms of the TGF-β are recognized, which are structurally similar in the c-terminal region. They are designated TGF-β1, 2 & 3, having the same functions in respect to their regulation of cellular growth and proliferation [1,3]. After being formed as secretory precursor polypeptide molecules (latent forms), they require proteolytic cleavage for activation to form a 25 KD homodimeric peptide [2,3]. The activators of the latent TGF-β are site – and function – dependent [6,7].

TGF-β1 is not only a potent stimulator of new connective tissue synthesis, but also the most potent inhibitor of smooth muscle proliferation [8]. It stimulates synthesis and deposition of extracellular matrix and its cell attachment, e.g. collagen, fibronectin, proteoglycans

and laminin [1,3,9]; a mechanism by which it might has a fibro pathogenic role [10]. It was reported that TGF-β1 was implicated in the pathogenesis of pulmonary and hepatic fibrosis [11,12]. Therefore, it may also be implicated in schistosomal-induced hepatic fibrosis [13].

On the other hand, TGF-β1 inhibits the proliferation and migration of vascular smooth muscle cell, promotes formation and secretion of protease inhibitors, has an anti-inflammatory function, suppresses the macrophage activation and leukocyte adhesion to endothelial cells and prevents intima formation [3,5, 14-19]; a mechanism by which it is atheroprotective and can play as a key inhibitor of atherogenesis [4,16,20].

Advanced schistosomal hepatic fibrosis was thought to be atheroprotective, since the incidence of atherosclerosis is not common in these patients [21]. There are many causes for this protection, where the associated hyperestrogenemia, hypolipidemia are the most important causes [21-24].

Aim of the work

The objective of this study is to estimate the serum level of transforming growth factor – beta1 in patients with schistosomal hepatic fibrosis, in an attempt to evaluate its fibro pathogenic and atheroprotective roles.

Material

Fifty subjects will be included in this study. They will be divided as follows:

1- A group of fifteen patients with schistosomiasis, who are negative for hepatitis B surface antigen and anti-HCV antibodies.
2- A group of fifteen patients with mixed infection of hepatic schistosomiasis and hepatitis C. All are negative for hepatitis B surface antigen.
3- A control group of twenty normal healthy volunteers of comparable age, sex and socioeconomic state as patients.

Methods

To all subjects the followings will be done:

1. Full history including smoking habits and thorough clinical examination including weight, height and blood pressure.
2. Calculation of body mass index.
3. Electrocardiography.
4. Abdominal ultrasonography.
5. X-ray on the chest.
6. Serodetection of HBs-Ag [25] and anti-HCV antibodies [26].
7. Estimation of the fasting serum levels of glucose, bilirubin, cholesterol (total and high- & low- density fractions) and triglycerides [27].
8. Determination of the serum activities of alanine and aspartate aminotransferases and alkaline phosphatase enzymes [27].
9. Estimation of serum amino terminal propeptide of type III procollagen [28].
10. Estimation of serum level of transforming growth factor-beta1 [29].

Results and discussion

The results obtained from the study will be tabulated, statistically analyzed, compared with other previous studies and discussed.

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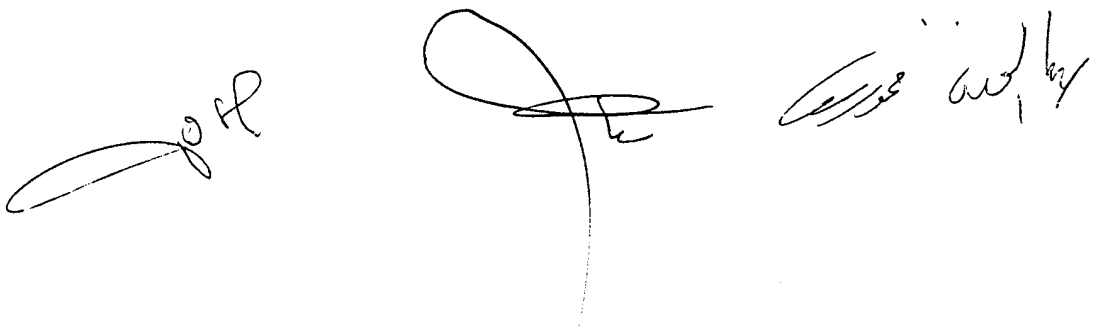

ARABIC SUMMARY

الملخص العربى

الملخص العربى

معامل التحول النموى - بيتا ١ فى مرضى التليف الكبدى البلهاريسى

يعتبر معامل التحول النموى- بيتا ١، واحد من عديدى الببتيدات والذى يجرى تكوينه فى معظم خلايا الجسم ويتكون من إفرازات خاملة تنتج عند الحاجة وتحتاج إلى الإنقسام البروتينى لتنشيطها ثم يتكون الببتيد المزدوج المتشابه وكتلته ٢٥ كيلو دالتون.

معامل التحول النموى بيتا١ ليس فقط محفزاً فعالاً فى تكوين النسيج الضام ولكن أيضاً أكثر فاعلية فى منع تكاثر العضلات الملساء. كما أنه ينبه إنتاج البروتينات فى النسيج المحيط بالخلايا وينظم إنتاج إنزيم الكولاجيناز وإنزيم الإلاستاز ومنشط للبلازميتوجن والأنزيمات الخاصة بتكسير البروتين فى هذا النسيج. وعلى الوجه الأخر، فإنه يمنع تكاثر وهجرة خلايا العضلات الملساء الخاصة بالأوعية الدموية، كما أنه يعمل كمضاد للالتهاب وكمثبط لنشاط كرات الدم البيضاء والتصاقها بخلايا الغشاء المبطن للأوعية الدموية ويمنع تكوين الطبقة الباطنة لها.

ولقد تبين من بعض الدراسات، أن معامل التحول النموى- بيتا١ كان له دور فى حدوث التليف الكبدى.

ومن المعروف، أن البلهارسيا الكبدية هى أكثر صور تليف الكبد انتشاراً فى مصر. ويعتبر التليف حول الوريد البابى هو النتيجة النهائية التى تنجم عن زيادة الأنسجة الضامة نتيجة للزيادة فى إنتاجها الحيوى والنقص فى تكسيرها أو بالعاملين مجتمعين.

إن زيادة إفراز الإستروجين وإنخفاض مستوى الدهون فى الدم وإنخفاض معدل التجلط وزيادة مؤشرات سيولة الدم تعتبر نتائج مشتركة عند المرضى بالتليف الكبدى البلهاريسى. مما يجعل هؤلاء المرضى أقل عرضة للإصابة بتصلب الشرايين.

والهدف من هذا البحث هو دراسة تأثير مستوى معامل التحول النموى- بيتا ١ فى الدم على مرضى البلهارسيا الكبدية فى محاولة لتقييم دوره فى نشأة التليف الكبدى وكذلك الحماية من تصلب الشرايين.

أجريت الدراسة على ثمانية وخمسين حالة قسمت إلى ثلاثة مجموعات:

- **المجموعة الأولى:** وتتكون من عشرين متطوعاً من الأصحاء (سبعة عشر من الذكور وثلاث من الإناث) من أعمار ومستويات اجتماعية واقتصادية متجانسة مع المرضى وذلك للمقارنة.
- **المجموعة الثانية:** وتتكون من ثمانية عشر مريضاً (ثلاثة عشر من الذكور وخمس من الإناث) تم تشخيص حالاتهم كمرضى بالتليف الكبدى البلهاريسى فقط بدون الإصابة بالفيروس الكبدى ب أو سى.
- **المجموعة الثالثة:** وتتكون من عشرين حالة (ستة عشرة من الذكور وأربع من الإناث) شخصت حالاتهم كمرضى بالتليف الكبدى البلهاريسى بالإضافة إلى إصابتهم بالفيروس الكبدى سى وكانوا جميعاً غير مصابين بالفيروس الكبدى ب. وجميع الحالات التى تم اختيارها غير مصابة بأى أمراض أخرى مثل الأورام السرطانية أو مرض السكر أو التهاب الكلى وغيرها، وهى من الأمراض التى قد تؤثر على مستوى معامل التحول النموى-بيتا ١ فى مصل الدم.

وقد كان جميع المرضى من غير المدخنين وغير مصابين بارتفاع ضغط الدم. وتم اختيارهم بناء على التاريخ المرضى والفحص الإكلينيكى والتحاليل المعملية. كما تم إجراء الفحوصات التالية على المجموعات قيد الدراسة:

- التاريخ المرضى، الفحص الإكلينيكى وحساب معامل كتلة الجسم والرسم الكهربائى للقلب والأشعة فوق الصوتية على البطن والأشعة السينية على الصدر.

كما تم عمل التحاليل التالية:

الكشف عن وجود كل من الإنتجين السطحى لفيروس الكبد "ب" والأجسام المضادة لفيروس الكبد "سى"، وكذلك قياس مستوى السكر الصائم ونسبة الصفراء الكلية والمباشرة والكوليسترول الكلى والدهون عالية الكثافة ومنخفضة الكثافة والدهون الثلاثية، كذلك نشاط أنزيمات المحولات الأمينية والفوسفاتيز القلوى فى مصل الدم، هذا بالعلاوة إلى تعيين مستويى ببتيد البروكولاجين ٣ ومعامل التحول النموى-بيتا ١.

وقد تبين فى هذه الدراسة أنه فى جميع المرضى، كان مستوى كل من معامل التحول النموى- بيتا١ وببتيد البروكولاجين ٣ مرتفعا عنه فى المجموعة الضابطة بصورة ذات دلالة إحصائية.

كما كان نشاط أنزيمات المحولات الأمينية والفوسفات القلوى أيضاً فى جميع المرضى أعلى بصورة ذات دلالة إحصائية عنه فى مجموعة الأصحاء.

ولقد تم تقييم معدل التليف الكبدى باستخدام الأشعة فوق الصوتية على الكبد، ولوحظ أن أعلى معدلات مستوى ببتيد البروكولاجين ٣ وجدت فى الحالات ذات درجات التليف المتقدم. كما لم يلاحظ أية علاقة واضحة بين مستوى معدل التحول النموى-بيتا ١ ودرجة التليف الكبدى.

أن مستوى معامل التحول النموى - بيتا ١ وببتيد البروكولاجين ٣ فى مجموعة المرضى المصابين بالتليف الكبدى البلهاريسى فقط، كانا أعلى نسبياً عنهما فى المجموعة الضابطة ولكن لم تصل هذه الزيادة إلى معدلات ذات دلالة إحصائية. وقد كان مستوى التحول النموى ـ بيتا ١ فى ثمانية من هؤلاء المرضى (٤٤,٤٪) وكذلك مستوى ببتيد البروكولاجين٣ فى أربعة منهم (٢٢,٢٪) أعلى من الحد الأقصى المحدد من المجموعة الضابطة.

وهذه النتائج تشير إلى وجود درجة بسيطة من التليف الكبدى مع ظهور معامل التحول النموى بيتا١. وبالإضافة إلى ذلك فإن خمسة من مرضى التليف البلهاريسى فقط، كان لديهم ارتفاع فى مستوى إنزيمات المحولات الأمينية وهو ما يشير إلى وجود تأثير طفيف على الخلايا الكبدية فى هؤلاء المرضى.

أما عن المرضى المصابين بالبلهارسيا الكبدية بالإضافة إلى إصابتهم بالفيروس الكبدى "سى" لقد أظهرت صور الأشعة فوق الصوتية على الكبد إصابتهم بصورة من التليف الكبدى المزدوج.

كما وجد فى هذه المجموعة أن مستوى كلاً من معامل التحول النموى- بيتا ١ وببتيد البروكولاجين ٣ كان مرتفعاً ارتفاعاً ذو دلالة إحصائية مقارنة بكل من المجموعة الضابطة ومجموعة المرضى المصابين بالتليف الكبدى البلهاريسى فقط. ولم يلاحظ فرق جوهرى فى معامل التحول النموى - بيتا ١ بالنسبة لدرجة التليف الكبدى ولكن كلما زادت درجة التليف كان مستوى ببتيد

البروكولاجين ٣ أعلى. وكذلك لم توجد علاقة ملموسة بين معامل التحول النموى –بيتا ١ أو ببتيد البروكولاجين ٣ فى هذه المجموعة من المرضى المصابين بالتليف الكبدى المزدوج.

بمقارنة المرضى ذوى مستوى ببتيد البروكولاجين٣ المرتفع فى المجموعتين، وجد أن مستوى إنزيم المحول الأمينى للألانين ومعامل التحول النموى– بيتا ١ كانا أعلى بصورة ذات دلالة إحصائية فى المصابين بالبلهارسيا الكبدية والفيروس الكبدى "سى" معا مقارنة بالمرضى المصابين بالبلهارسيا الكبدية فقط. وهذه النتائج تفيد بوجود تأثير أعلى على الخلايا الكبدية وعلى نشاط التليف الكبدى عند إصابة مرضى البلهارسيا الكبدية بالفيروس الكبدى "سى".

ومن جهة أخرى، فإنه قد تم بحث العلاقة بين مستوى معامل التحول النموى – بيتا ١ وتصلب الشرايين حيث أنه كان من المعتقد أن المرضى بالتليف الكبدى البلهاريسى لديهم وقاية من تصلب الشرايين.

لقد وجد فى كل المرضى الذين تمت دراسة حالتهم مجتمعين أو فى كل مجموعة على حدة، أن نسبة الكوليسترول الكلى والدهون منخفضة الكثافة، أقل من مثيلاتها بالمجموعة الضابطة. وبتقسيم نمط الدهون وفقا لقيم حدود المجموعة الضابطة إلى مستوى منخفض ومستوى عال، وجد أن عدد المرضى الذين يعانون من ارتفاع مستوى الدهون قليل الحدوث وخصوصا فى المجموعة المصابة بالتليف الكبدى البلهاريسى. وعلى العكس وجد أن عدد المرضى الذين كان لديهم انخفاض فى مستوى الدهون كثير الحدوث وخصوصا فى المجموعة المصابة بالتليف الكبدى البلهاريسى والفيروس الكبدى "سى" معا. وهذه النتائج تفسر عدم انتشار الإصابة بتصلب الشرايين فى المرضى المصابين بالتليف الكبدى البلهاريسى وحدها أو المصاحبة للإصابة بالفيروس الكبدى "سى". كما لم يوجد اختلاف ذو دلالة إحصائية فى مستويات معامل التحول النموى – بيتا ١ فى كلا من مجموعتى المرضى عند تقسيمهم حسب مستوى الدهون لديهم.

ومن ناحية أخرى لقد وجد ارتفاع نسبى فى معامل التحول النموى – بيتا ١ فى المرضى ذوى المستويات المنخفضة الدهون (غير الخطرة). ولقد أصبح هذا الارتفاع ذو دلالة إحصائية فى

مجموعة المرضى المصابة بالتليف الكبدى البلهاريسى بالإضافة إلى إصابتهم بالفيروس الكبدى "سى".

ومن النتائج السابقة يمكن أن نستخلص الآتى:

- لم يظهر تغيير فى مستوى معامل التحول النموى- بيتا ١ فى مرضى التليف الكبدى البلهاريسى بينما زاد هذا المستوى فى حالات التليف الكبدى البلهاريسى المصاحب للإصابة بالفيروس الكبدى "سى".
- وقد كانت الزيادة فى مستوى ببتيد البروكولاجين٣ ذات دلالة إحصائية. كما أن مستواه كان يرتفع كلما ازدادت درجة التليف الكبدى. مما يشير إلى وجود علاقة بين درجة ارتفاعه وبين درجة التليف الكبدى.
- لا توجد علاقة بين مستوى معامل التحول النموى بيتا ١ والتليف الكبدى، وعلى ذلك فإن معامل التحول النموى بيتا ١ وحده لا يعتبر مؤشراً جيداً لقياس درجة التليف فى حالات التليف الكبدى البلهاريسى الصرف.
- ويعتبر مستوى ببتيد البروكولاجين ٣ مقياساً أفضل لتحديد درجة التليف فى مثل هذه الحالات، حيث أنه يزيد كلما زادت درجة التليف.
- إن معدل الزيادة فى مستوى معامل التحول النموى – بيتا ١ فى مرضى التليف الكبدى البلهاريسى قد يشير إلى وجود عدوى مصاحبة للإصابة بالفيروس الكبدى "سى"، خاصة إذا صاحب ذلك زيادة فى مستوى ببتيد البروكولاجين ٣.
- إن انخفاض معدل الإصابة بتصلب الشرايين فى مرضى التليف الكبدى البلهاريسى يمكن إرجاعه إلى وجود انخفاض فى مستوى الدهون فى الدم وكذلك إلى الارتفاع فى مستوى معامل التحول النموى- بيتا ١ معاً.

وأخيراً فمن النتائج السابقة فإنه من الممكن افتراض وجود دور لمعامل التحول النموى – بيتا ١ فى حدوث واستمرارية التليف الكبدى البلهاريسى.

ومن جانب أخر فإنه بالرغم من عدم إمكانية إثبات دور لمعامل التحول النموى- بيتا ١ فى الوقاية من تصلب الشرايين فى هؤلاء المرضى فإن هذا الدور لا يمكن إغفاله.

المشـــــرفون

أ.د. / صفاء عبد الرحمن الحفنى

أستاذ الباثولوجيا الإكلينيكية

معهد البحوث الطبية

جامعة الإسكندرية

أ.د. / ثناء فتحى مغازى

أستاذ الباثولوجيا الإكلينيكية

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جامعة الإسكندرية

د. / محمود سعد رجب

أستاذ مساعد الباثولوجيا الكيميائية

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جامعة الإسكندرية

د. / جمال أحمد أمين

أستاذ مساعد الأمراض الباطنة

معهد البحوث الطبية

جامعة الإسكندرية

جامعة الإسكندرية

معهد البحوث الطبية

الدراسات العليا

موافقة العميد على تشكيل لجنة الحكم بالتفويض فى ٢٠٠٢/٣/٧

أعضاء اللجنة

الاسم	الوظيفة	
١- أ.د/ صفاء عبد الرحمن الحفنى	أستاذ الباثولوجيا الإكلينيكية بالمعهد	(مشرفان وممتحنان داخليان)
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٢- أ.د/ منى محمد أباظه	أستاذ الباثولوجيا الكيميائية بالمعهد	(ممتحن داخلى)
٣- أ.د/ صلاح أحمد مرزوق	أستاذ الباثولوجيا الإكلينيكية بكلية طب الإسكندرية	(ممتحن خارجى)

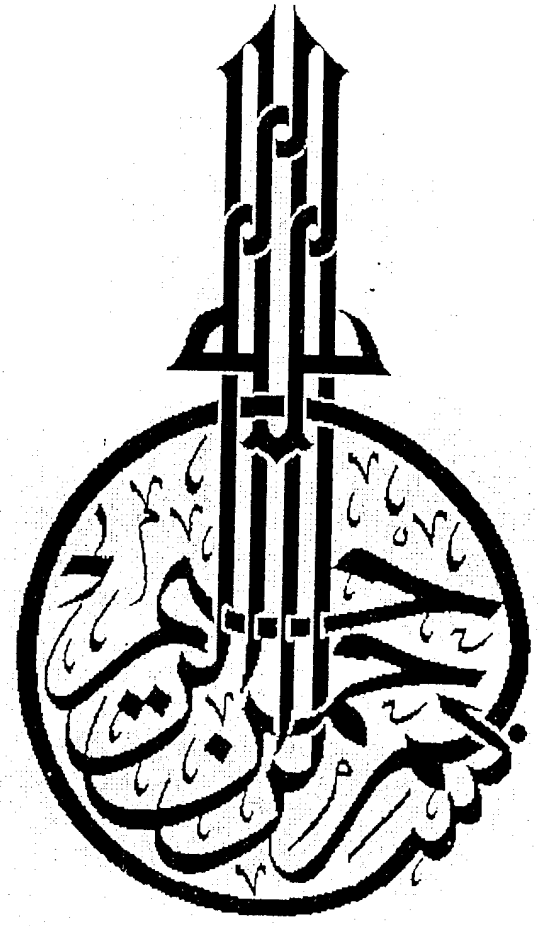
موافقة مجلس المعهد على منح الدرجة فى ١٤ / ١ /٢٠٠٣ بتقدير جيد جداً

يعتمد،

وكيل المعهد

للدراسات العليا والبحوث

(أ.د.)



قالوا سبحانك لا علم لنا إلا ما علمتنا
إنك أنت العليم الحكيم

البقرة آية ٣٢

صدق الله العظيم

معامل التحول النموى - بيتا ١
فى مرضى التليف الكبدى البلهاريسى

رسالة

مقدمة إلى معهد البحوث الطبية - جامعة الإسكندرية
إيفاءا جزئياً لشروط الحصول على درجة

الماجستير في

الكيمياء الباثولوجية

مقدمة من

شيماء سمير السيد بدوى

بكالوريوس الطب والجراحة - جامعة الإسكندرية - ١٩٩٦

معهد البحوث الطبية
جامعة الإسكندرية
٢٠٠٢