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Full Length Research Paper

Plasma nitric oxide metabolites and endothelin-1 concentrations in schistosomal porto-pulmonary hypertensions

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Abstract

The vasodilator nitric oxide (NO) and the vasoconstrictor endothelin-1(ET-1) are the principal vasoreactive regulators of the vascular tone and regional blood flow (portal venous and pulmonary arterial systems). Their syntheses are anatomically dependent processes and their circulating concentrations among schistosomal porto-pulmonary hypertensives are not yet elucidated. Assessment of plasma nitric oxide metabolites (nitrite and nitrate) together with endothelin-1 levels in patients with schistosomal porto-pulmonary hypertension. One hundred and fifty patients with schistosomiasis were withdrawn from the Internal Medicine, Tropical Medicine and Cardiology Outpatient Clinics, Mansoura University Hospitals. Of thems 25 patients had simple intestinal schistosoma mansoni infestations without any complications, 50 patients suffered from bilharzial portal hypertension, 25 patients had hepatospleenomegallies, ascites and intrahepatic presinusoid and sinusoid fibrosis and 50 patients suffered from both bilharzial portal vein and pulmonary artery hypertensions. At the same time, a healthy reference group comprized 25 clinically and laboratory normal individuals and free of schistosomiasis were similarly investigated. They were highly matched for age, gender and body mass index with the diseased groups. 8.0 ml fasting venous blood sample were obtained from every subject (patient or control). Half of which was left to clot and the separated serum was used for determination of routine kidney and liver functions tests. The other half of each sample was added to EDTA (1.0mg EDTA/1.0 ml blood) and the separated plasma was used for assay of NO_2 and NO_3 as well as ET-1 concentrations. Plasma nitrite, nitrate and total nitrates (NOx) mean concentrations (umol/L) were significantly lower in combined schistosomal portal vein and pulmonary artery hypertensions than their correspondings in healthy control. On the other hand, the mean plasma concentrations of NO_2, NO_3 and total NOx in portal hypertensive group were significantly higher than their correspondings of the healthy control, simple uncomplicated schistosomal and pulmonary + portal hypertensive groups. At the same time, significantly higher plasma ET-1 concentrations (pg/ml) in different hypertensive patients' groups were found in comparison to normal control and simple intestinal schistosomiasis values. This can be attributed to a high ET-1 synthesis and release rate into the portal/pulmonary blood flow than the corresponding normal level. Plasma nitric oxide metabolites concentrations were significantly subnormal while ET-1 concentrations were significantly higher in schistosomal portal + pulmonary hypertensive patients in comparison to healthy control values. On the other hand, schistosoma mansoni inducing portal hypertension only was associated with elevated plasma NO_2, NO_3, NOx and of ET-1 concentrations than the corresponding normal values. So, NO synthesis rate is site dependent.

Keywords: Schistosomal hepatospleenomegally, Total nitrate, Endothelin-1.

INTRODUCTION

Schistosoma mansoni is a digeneic intravascular parasite. Infection by this helminth is endemic in many equatorial tropical and subtropical countries including Egypt. The parasite lives normally within the porto-

mesenteric venous system of man. Schistosomal eggs deposited by adult female worms pass readily to the intestinal lumen, but some ova can pass upward through the portal vein to the pulmonary artery where they are trapped in either the liver or the lung, inducing variable pathology that can run from chronic inflammations or granulomas to severe hepatic portal vein or pulmonary artery stenosis due to reactive fibrous tissue formed around these vessels (Sheta and El-Saadany, 2006; Andrade, 2009).

Hepatic-portal hypertension manifested by hemodynamic changes in the portomesenteric venous circulation due to difficult portal blood outflow from the portal vascular bed second to increased vascular resistance and expanded blood volume in this system. The underlying lesion(s) that induce portal hypertension is either a prehepatic, an intrahepatic or a post hepatic anomaly. Schistosomal fibrosis is a presinusoid while viral cirrhosis is a sinusoid one, both of them belong to the intrahepatic induced forms (Petruff and Chopra, 2004; Shah, 2007;Cichoz-Lach et al., 2008; Muti et al., 2012).

Normally vasodilators [nitric oxide (NO) prostaglandins, tumor necrosis factor-α and carbon monoxide] increase hepatic portal blood flow, while vasoconstrictors [endothelin-1 (ET-1) and cyclo-oxygenase-derived prostaglandins] induce the reverse. Imbalance of both vasoactive groups determines the net response of the vascular bed as either vasodilatation or vasoconstriction. So, ET-1 over production and/or NO impaired release increase the vascular tone in the presinusoid/sinusoid hepatic areas (De Franchis, 2005). However, in schistosomal portal hypertension, NO is not produced in the presinusoid sector which increases vascular resistance and hyperkinetic circulation in this area (Petruff and Chopra, 2004; De Franchis, 2005; Shah, 2007; Muti et al., 2012). The vascular endothelium, the inner cellular lining of blood vessels, produces these powerful vasoactive substances in response to many vascular physical factors such as blood flow, oxygen tension and regional blood pressure.

Pulmonary artery hypertension is an elevated pulmonary arterial pressure due to an increased resistance to blood flow (Andrade, 2009). While core pulmonale is the pathologic lung dysfunction due to hypertension of the right side of the heart. It is found in about 25% of bilharzial pulmonary hypertensive patients (Hampl and Herget, 2000; Nauser and Stites, 2003; Robotham, 2003).

Nitric oxide gas is decomposed regionally (Arkenau et al., 2002; Gatta et al., 2008) and very rapidly ($\leq$10 secs) after its release and induction of biological activities (Abo-Shousha et al., 1999). Therefore stable NO metabolites (NO_2 and NO_3 and the sum of both NO_x) are commonly used as surrogate markers of NO production (Petruff and Chopra, 2004; De Franchis, 2005; Shah, 2007; Muti et al., 2012). While schistosomal portal vein hypertension is commonly associated with higher plasma NO_x concentrations (Abo-Shousha et al., 1999; Arkenau et al.,2002; Parvu et al., 2005; Gatta et al., 2008), schistosomal pulmonary hypertension frequently shows lower plasma NO_x levels (Ghofrani et al., 2004; Parvu et al., 2005; El-Kannishy et al., 2007) in comparison to the healthy control values. However, in both conditions, plasma ET-1 concentrations are oftenly higher than the healthy (Kapoor et al., 2003; Kandil et al., 2004; Neuhofer et al., 2006; Watanabe et al., 2007).

In view of the above data, the present study was designed to evaluate the circulating plasma NO metabolites (NO_2 and NO_3) and ET-1 concentrations in patients with schistosomal porto-pulmonary hypertensions.

Subjects

This study was conducted on 150 adult patients (123 males+27 females) with schistosoma mansoni affections. They attended to the Internal and Tropical Medicine and Cardiology Outpatient Clinics of Mansoura Faculty of Medicine, Egypt. All ill participants had schistosoma mansoni ova in their stool or rectal snip samples or were positive for schistosoma hemagglutination-inhibition test (titre: $\geq$1/600). They were categorized into (Table 1):

1. Twenty five adult patients (16 male and 9 females) complaining of simple intestinal schistosoma mansoni infection.
2. Fifty cases suffered from schistosomal portal hypertension with moderate spleenomegally but no ascites.
3. Fifty cases had chronic schistosomal porto-pulmonary hypertensions manifested clinically as core pulmonale combined with portal hypertension (their spleens were moderatly enlarged than normal).
4. Twenty five patients each suffered from large spleen, shrunken liver, portal hypertension, ascites and intrahepatic presinusoid and sinusoid fibrosis.
5. A control group comprized 25 clinically healthy nonschistosomal individuals who were matched for age, body mass index and gender with the patients' groups.

Any patient with one or more of the following stigmata was excluded from the study. Extremities of age, obesity, severe smoking, viral B or C hepatitis (HBs-Ag and HBc-IgM and HCV negative testing), cardiac, renal, respiratory, endocrinal or cerebro-spinal disease, antibilharzial therapy within the last 6.0 months and intake of diets (spinach, beats vanilia or banana) or drugs (nitrate therapy).

All patients and healthy control individuals agreed to participate in the present study by giving signed informed consents. Also the scientific commete of Mansoura Faculty of Medicine accepted the study protocol.

Clinical examinations and biochemical and ultrasonographic investigations were done for all included bilharzial subjects.

Blood samples

- Five mls 12 hrs-fasting venous blood samples were withdrawn slowly but without stasis or frothing from every subject (patient and control) and put into a 5-ml vacutainer tube containing 1.0 mg EDTA/1.0ml blood to be used for nitrite, nitrate and ET-1 determinations. Tubes were immediately transported on ice to the laboratory dependant where blood was centrifuged and the unhemolyzed plasma was separated to be use as follow:

(1) (a) After ultra filtration through a 1000 MMWCO filter (to get protein free plasma and thus avoid artificially high NO_2 levels), each plasma sample was divided into 2.0x0.75 ml aliquots, placed in cryovials and stored at -70°C till NO metabolites analyses. Determinations were performed in duplicates. Native NO_2 was photometrically determined by the colored azo dye product of the Griess reaction that absorbs visible light at 540 nm (Moshage et al., 1995; Viinikka, 1996; Li and Billiar, 1999) using assay design, Inc. 800 Technology Drive. Ann Arbor, MI 48108 USA. Meanwhile, the other stable end product of NO i.e. nitrate ($NO3^-$), can not be detected by Griess reagent treatment of the plasma samples with nitrate reductase before analysis induced enzymatic conversion of $NO3^-$ to $NO2^-$ which was then ready for determination.

(b) At the same time, 2.0x0.75 ml plasma aliquots were stored in cryovials at -70ºc till ET-1 determination. ET-1 assay in plasma was done by enzyme immunometric assay (EIA) (Tijssen, 1985) (Byproducts of Assay Designs. Inc: 800 Technology Drive Ann arbor MI: 48108 USA).

(2) Another 5 ml blood sample was withdrawn from every patient for performance of the essential routine investigations such as blood cells counts and plasma glucose, serum creatinine and selected liver function tests. The routine serum biochemical analyses were performed using the corresponding reagents and methods of Human Products, Germany.

Statistical analysis

SPSS for windows version 11 (SPSS Inc., Chicago-USA) was used for calculation of statistical parameters. Data being parametric, results were presented as mean $\pm$ standard deviation. Statistical results of each patients group were compared with their corresponding for healthy reference subjects as well as with other diseased groups to get t test and P values. In addition, correlation coefficients were determined. Finally, the significance for any of these tests was set at $P \leq 0.05$.

RESULTS

The obtained results were shown in the following tables:

DISCUSSION

Nitric oxide is a protective factor that mediates effective biological functions (vasodilator induction and apoptosis inhibition) but can be cytotoxic when oxidized to free nitrogenous radicals. The ratio percent of the two major stable breakdown by products [nitrite (NO_2) and nitrate (NO_3)] of their parent NO vary from tissue to tissue and from organ to organ. In consequence, both metabolites should be measured simultaneously in every study (Robotham, 2003; Parvu et al., 2005; El-Kannishy et al., 2007).

In the present study, there were significantly higher concentrations of plasma NO_2, NO_3 and NO_x in patients with schistosomal hepatospleenic (SHS) portal hypertension compared to the corresponding healthy control and simple intestinal schistosomiasis values (Table 4). There is a general agreement that the circulating levels of NO_x in schistosoma mansoni produced portal hypertension and its sequelae (fibrosis, ascites, gastric varices and encephalopathy) were higher than normal control but the magnitudes of such changes were variable in different organs (Abo-Shousha et al.,1999; Kandil et al., 2004; Parvu et al., 2005).

Portal venous inflow in schistosomal hepatospleenomegally (SHS) was increased due to the hyperdynamic circulatory state and increased plasma volume within the splanchnic area (Petruff and Chopra, 2004; Shah, 2007; Muti et al., 2012). The natural decrease of NO production within the hepatic tissue increases sinusoidal tone and decreases liver perfusion ((De Franchis, 2005; Gatta et al., 2008; Muti et al., 2012). Portal hypertensive patients in this study had almost normal renal functions (Table 3) (the main route for excretion of NO by products), the underlying cause of the significantly higher than normal NO_x values (Table 4) was most possibly an excess NO portomesenteric production rather than an impaired NO_x renal excretion.

In schistosomal hepatic fibrosis group with complications (Table 4), plasma NO metabolites and ET-1 peptide concentrations were similar to those detected in uncomplicated portal hypertensive patients. So, these vasoactive reactions were not dependant on the stage of bilharzial hepatic pathology.

Decreased NO production has been observed within fibrotic livers and is associated with constriction of hepatic sinusoids and increased hepatic resistance to portal blood flow. Alternatively, an increased NO within the intestinal mesenteric area could cause presinusoid dilation and increase of portal blood flow. These opposing events introduce a challenge in the ability to target eNOS in a portal hypertension treatment paradigm. Nonspecific NOS inhibitors prevent systemic and mesenteric hyperemia and abrogate the development of portal hypertension (Theodorakis et al., 2009; Bari and Garcia-Tsao, 2012).

Prolonged elevation of pulmonary arterial pressure

Table 1. Bsaeline clinical data of bilharizial pulmonary and/or portal hypertensives

Data	Percentage %
Dyspepsia in portal hypertensives:	80
Dyspnea in pulmonary hypertensives	80
Pallor	100
Jaundice	0
Edema in lower limbs	0
Congested neck veins	30
Hepatosplenomegally	100
Fragile gastro-esophageal vascular collateral in hepatic fibrosis cases**	68
2nd left space pulsation and dullness	100
Diastolic murmur	60
X-ray pulmonary dilatation	60
HBV and HCV diagnostic tests	Negative
IHA test for bilharziasis	Positive at 640 or more

•* The spleens were large, (the single most important diagnostic sign of portal hypertension), their edges were firm and size beared moderate relation to the portal pressure. At the same time, the livers were slightly smaller than normal. Their consistency was firm.

•** Late stages of periportal vascular fibrosis were commonly associated with retention of blood within the portal area, formation of fragile gastro-esophageal collateral circulations that directly connect the portal blood vessels with the general circulation bypassing the liver and inducing hematemesis, melena and anemia, refractory ascites that was resistant to treatment, altered endothelial vasoactive products and/or hepatic encephalopathy.

Table 2. Age, body mass index and gender data in the studied groups.

Items	Age (Years)	Body mass index /Kg/m^2	Sex	
			Female No	Male No
Normal Control (25 individuals)	38.0±6.0	24.8±3.1	6	19
Portal hypertensives (50 cases)	41.9±5.1	22.2±4.5	8	42
Pulmonary hypertensives (50cases)	40.5±5.4	23.0±3.5	7	43
Hepatic fibrosis (25 cases)	42.8±5.9	22.7±4.2	6	19

No significant difference between the different groups.

Table 3. Selected liver and renal function tests (mean±SD) in the different studied groups

Data	Bilirubin (mg/dL)	ALT (U/L)	Albumin (g/dL)	Prothrombin Activity %	S.Creatinine mg/dL
Control (25 cases)	0.69±0.2	22.5±4.0	4.2±0.2	98.2±4.0	0.7+0.2
Portal hypertensive gr (50 cases)	1.4±0.3	55.3±14.3	3.9±0.3	70.0±9.1	1.0±0.3
Pulmonary hypertensive gr (50 cases)	1.1±0.3	48.2±7.8	3.9±0.3	74.0±10.5	0.9±0.4
Hepatic fibrosis (25 cases)	1.4±0.4	53.5±8.3	3.7±0.4	66.2±11.5	1.1±0.4

* Significant difference from healthy control ($p<0.01$)

Table 4. Plasma stable NO metabolites: (NO_2, NO_3 and NO_x) in the different studied groups.

Items	NO_2 (µmol/L)	NO_3 (µmol/L)	NO_x (µmol/L)	Endothelin-1 (pg/ml)
Normal control	11.4±2.5	24.8±5.3	36.2±6.3	2.35±0.8
Simple intestinal schist.	12.5±2.7	25.5±6.2	38.0±6.6	2.5±0.8
Portal hypertensive gr (50 patients)	17.4±3.5*	38.9±6.2*	56.3±10.7*	3.5±0.9*
Pulmonary hypertensive gr (50 patients)	8.8±4.9*	20.4±7.0*	29.2±8.4*	3.6±1.3*
Hepatic fibrosis gr (25 patients)	15.4±5.0*	37.0±16.5*	52.4±20.1*	3.64±1.6*

* Significant difference from healthy control ($p<0.01$)

Table 5. Correlation between nitrite, nitrate, total nitrate and ET-1 concentrations in normal, portal hypertensive and portopulmonary hypertensive groups

Data	NO_2^- VS NOx	NO_3^- VS NOx	NOx VS ET-1
Normal control group:			
r	0.56	0.77	0.90
p	<0.05	<0.01	>0.005
Portal hypertension group:			
r	0.66	0.84	0.68
p	<0.05	<0.01	<0.01
Pulmonary hypertension group:			
r	0.65	0.93	0.73
p	<0.01	<0.01	<0.01

There were significant positive correlations between plasma concentrations of NO_3 and NO_x in all studied groups (diseased or healthy) and between NOx and ET-1 in portal hypertensive group. Alternatively, significant negative correlations were observed between plasma NO metabolites and ET-1 concentrations in pulmonary + portal hypertensives.

with an increased blood flow resistance (Andrade, 2009) can induce core pulmonale due to hypertension in the right side of the heart (Hampl and Herget, 2000; Nauser and Stites, 2003; Robotham, 2003). The present study demonstrated significant decrease in the mean concentrations of plasma nitrite, nitrate and total nitrates in patients with bilharzial porto-pulmonary hypertension when compared with the corresponding normal control values (Table 4). This may be due to rapid expiration of the produced NO gas particularly that these patients suffered from dyspnea-hyperapnea (Table1). Similar data had been recently reported (El-Kannishy et al., 2007).

Ozkan et al., 2001 hypothized an increase of the circulating NO in pulmonary hypertension. Clini and Ambrosino 2002 proved that pulmonary hypertension was associated with decrease in endothelial nitric oxide production both at rest and during exercise. Cella et al., 2001 found that circulating NO level was significantly subnormal in patients with pulmonary hypertension compared with those of healthy control subjects.

Endothelin-1 is a 28 polypeptide that has potent and distinct vasoconstrictive properties and is synthesized by the vascular endothelium in different tissues. ET-1 secretion is stimulated by a variety of stimuli, including hypoxia, dehydration, endotoxin, shear stress and baroreceptor activation. It is also influenced by several vasoactive substances such as catecholamines, rennin-angiotensin system, arginine-vasopressin, and prostacyclin. Endothelin peptides and their cognate receptors are causally involved in the pathophysiology of pulmonary arterial hypertension, portal hypertension and hepatic fibrotic remodeling (Hoeper and Krowka, 2004; Ramadori et al., 2008).

In the present study (Table 4), plasma ET-1 concentrations were significantly increased in schistosomal portal hypertension irrespective of pulmo-

nary NO production state. Similar results were observed in cases with hepatic fibrosis (Table 4) which confirmed some previous results (Kandil et al., 2004; Hoeper and Krowka, 2004). Active vasoconstrictors mainly endothelin-1 counteract NO vasodilation activity. The relative dysfunction of both these vasoreactive substances determines the net response of the vascular bed in the form of either vasodilation or vasoconstriction (Shah, 2007). On the other hand, the increase in secretion of endothelium-1 and removal of nitric oxide contribute toward the efficacy of diaspirin cross linked hemoglobin in hemorrhage (Gulati et al., 1996).

REFERENCES

Abo-Shousha S, Khalil SS, Rashwan EA (1999). Oxygen free radical and nitric oxide production in single or combined human schistosomasis and fascioliasis. J. Egypt. Soc. Pparasitol; 29:149-56.

Andrade ZA (2009). Schistosomiasis and liver fibrosis. Parasite Immunol. 31: pp 656-663.

Arkenau HT, Stichenoth DO, Frolich JC, Manns MP, Boker KH (2002). Elevated NO levels in patients with chronic liver disease and cirrhosis correlate with disease stage and parameters of hyperdynamic circulation. Z Gastroenterol, 40:907-913.

Bari K, Garcia-Tsao G (2012). Treatment of portal hypertension. World Gastroenterol, 18:1166-1175.

Cella G, Bellotto F, Tona F, Sbarai A, Mazzaro G, Motta G, Fareed J (2001). Plasma markers of endothelial dysfunction in pulmonary hypertension. Chest; 120: 1226-1233.

Cichoz-Lach H, Celinski K, Slomka M, Kasztelan-Szezerbinska B (2008). Pathophysiology of portal hypertension. J. Physiol. Phrmacol. 59: Supp:231-238.

Clini E, Ambrosino N (2002). INOC Italian Nitric Oxide Club: Nitric oxide and pulmonary circulation. Med. Sci. Monit. RA 8: 178-182.

De Franchis R (2005). Envolving consensus in portal hypertension. Report of the Baveno IV consensus workshop on methodology, diagnosis and therapy of portal hypertension. J. Hepatol. 43:167-176.

El-Kannishy MH, El-Demerdash FM, Abd El-Gawad S.SH, Daoud EM, El-Shahat N, Soliman AW (2007). Plasma nitric oxide concentration in schistosomal pulmonary hypertension. Egypt Heart J. 59:63-9.

Gatta A, Bolognesi M, Merkel C (2008). Vasoactive factors and hemodynamic mechanisms in the pathophysiology of portal hypertension in cirrhosis. Mol. Aspects Med. 29:119-129.

Ghofrani HA, Pepke-Zaba J, Barbera JA, Channick R, Keogh AM, Gomez-Sanchez MA, Kneussl M, Grimminger F (2004). Nitric oxide pathway and phosphodiestrase inhibitors in pulmonary arterial hypertension. J. Am. Coll. Cardiol. 43 (Suppl 12): S68-72.

Gulati A, AP Sen, Kumar A, Tyagi MG (1996). Increase in endothelin and removal of nitric oxide (NO) contribute toward the efficacy of diaspirin crosslinked hemoglobin in hemorrhaged rats. Artif Cells Blood Substit Immobil Biotechnol, 24:345.

Hampl V, Herget J (2000). Role of nitric oxide in the pathogenesis of chronic pulmonary hypertension. Physiol. Rev. 80: 1337-1372.

Hoeper MM, Krowka MJ, Strassburg CP (2004). Portopulmonary hypertension and hepatopulmonary syndrome. Lancet, 363:1461-1466.

Kandil M, Magour G, El-Gindy M, Abdel Baky M, Fadaly G, Abdel Moety H (2004). Study of portal and systemic levels of nitric oxide, endothelin and procellagen III peptide in chronic liver disease in Egypt. J. Med. Res. Institute, 25: 178-192.

Kapoor D, Redhead DN, Hayes PC (2003). Systemic and regional changes in plasma endothelin following transient increase in portal pressure. Liver transplant; 9:32-9.

Li J, Billiar T (1999). Determinants of nitric oxide protection and toxicity in liver. Am. J. Gastroenterol. 276(5ptl):G1073-G82.

Moshage H, Kok B, Huizenga JR, Janwn PL (1995). Nirite and nitrate determinations in plasma: a critical evaluation. Clin. Chem. 41:892-896.

Muti LA, Sparchez Z, Iobagiu S, Acalovschi M (2012). The etiology of non-cirrhotic portal hypertension. A study of 105 consecutive patients. Clujil Medical. 85:200-206.

Nauser TD, Stites SW (2003). Pulmonary hypertension: New prospectives. Congest Heart Fail, 9: 155-162.

Neuhofer W, Gülberg V, Gerbes AL (2006): Endothelin and endothelin receptor antagonism in portopulmonary hypertension. Eurp. Clin. Invest. 36 (suppl 3): 54-61.

Ozkan M, Dweik RA, Laskowski D, Arroliga AC, Erzurum SC (2001). High level of nitric oxide in individuals with pulmonary hypertension receiving epoprostenol therapy. Lung 179: 233-243.

Parvu A, Ngrean V, Pleca-Manea L, Cosma A, Draghic A (2005). Nitric oxide in patients with chronic liver diseases. Romania J. Gastroenterol. 14:225-230.

Petruff CA, Chopra S (2004). Classification of portal hypertension. In Handbook of liver disease. Ls Friedman, Ls keeffe EB (eds). Philadelphia, Churchill Livingstone, pp: 264-167.

Ramadori AG, Moriconi F, Malik I, Dudas J (2008). Physiology and pathophysiology of liver inflammation, damage and repair. J. Physiol. Pharmacol. 59(supplI): 107-117.

Robotham JL (2003). Schistosomal cor pulmonale: A fluke in the Fas lane?. Respiration, 70: 569-571.

Shah V (2007). Molecular mechanisms of increased intrahepatic resistance in portal hypertension. J. Clin. Gastroenterol. 41 (suppl 3) S259-61.

Sheta EA, El-Saadany SH (2006). Schistosomiasis. Tanta Medical Sciences,1:1-10.

Theodorakis NG, Wang YN, Wu JM, Maluccio MA, Sitzmann JV, Skill NJ (2009). Role of endothelial nitric oxide synthase in the development of portal hypertension in the carbon tetrachloride-induced liver fibrosis model. Am. J. physiol. Gastrointest Liver Physiol. 297:G792-G799.

Tijssen P (1985). Practice and theory of enzyme immunoassay, Elsevier Amesterdam,

Viinikka L (1996) Nitric oxide as a challenge for the clinical chemistry laboratory. Scand J. Clin. Lab. Invest. 56:577-581.

Watanabe N, Takashimizu S, Nishizaki Y, Kojima S, Kagawa T, Matsuzaki S (2007). An endothelin A receptor antagonist induces dilation of sinusoidal endotheilial fenestrae: implications for endothelin-1 in hepatic microcirculation. J Gastroenterol, 42: 775-782.