



Study the Activity of Arginase Enzyme and Biochemical Parameters in Patients of Typhoid Fever

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Abstract

Typhoid fever causes significant biochemical changes, serum biochemistry was studied to investigate the correlation of these factors to typhoid fever. Total 100 subjects were studied, 35 healthy individuals and 65 typhoid patients. Blood samples were collected in a gel and anticoagulant tubes from Azadi Teaching Hospital, Kirkuk, Iraq. In this study, Widal test was used to test samples. High level of Arginase activity ($P \leq 0.01$) were observed in typhoid patients. And a number of biochemical parameters were measured, a Significantly increased at a level of probability ($p \leq 0.01$) in the (Alanin Aminotransferase) ALT, (Aspartate transaminase) AST, Urea, Creatinine, Uric acid, Triglyceride, Very low density lipoprotein (VLDL) for patients with Typhoid fever as compared with the control group. And a Significantly decreased at a level of probability ($p \leq 0.01$) in the Cholesterol, Total protein, Albumin for patients with Typhoid fever as compared with the control group. And the level of Globulin ($P \leq 0.05$) was not recorded significant differences.

Keywords: Typhoid fever ; Arginase activity ; ALT ; AST; Urea ; Creatinine ; Uric acid ; T.protein ; Albumin ; Globulin ; Cholesterol ; Triglyceride ; VLDL.

1. Introduction

Enteric fever is a fundamental disease caused by the human adjusted pathogens *Salmonella enterica* serotype Typhi (*S. Typhi*) and *S. Paratyphi* A, B, and C. These living beings are essential reasons for febrile disease among swarmed and devastated populaces with lacking sanitation who are presented to risky water and nourishment, and furthermore represent a hazard to voyagers going to endemic nations [1].

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Typhoid fever is broadly perceived as a noteworthy general medical issue in creating nations Rate of typhoid fever that been set up as roughly as 22 million cases with at slightest 200,000 passing happening yearly [2-4]. Typhoid fever is caused by *Salmonella Enteric a SerovarTyphi*, a gram-negative bacterium [5]. The creature is transmitted by the fecal-oralcourse; subsequently, the sickness witch is frequently connected with poor sanitation and cleanliness [4,6]. Typhoid fever shows with fever, cerebral pain, stomach torment, gastrointestinal side effect like anorexia sickness, regurgitating ,and stoppage. The basic sings like stomach delicacy may create [7].

Arginase enzyme belongs to the urea hydrolase family enzymes. The arginase Arg (EC.3. 5. 3.1), has 105.kDa molecular weight, has optimal pH= 9-9.5, PI= 5.9,catalyzes the final step in the urea cycle, a series of biochemical reactions in mammals in which the body is disposed of harmful ammonia. Specifically, arginase-convert L-arginine into L-ornithine and urea[8].

In most mammals,thereare two isozymes of arginase enzyme exist; the first, Arginase I functions in the urea cycle and is located in the cytoplasm of the liver. The second isozyme, Arginase II,it has been implicated in the regulation of the arginine/ ornithine concentrations in the cell. It is located in mitochondria in several tissues in the bodywith most abundance in the kidney [9].

2. Materials and methods

2.1 Selection of patients

This study was conducted in the Department of Biochemistry lab in Kirkuk university, Iraq. It included 65 patients diagnosed with typhoid fever and 35 controls were also involved in the study.

All were informed regarding the study and written consent was obtained. General information such as name, age, gender etc was recorded in case of history performa. Blood samples were collected in a gel and anticoagulant tubes from normal and typhoid infected patients.

2.2 Serological Technique

High purity chemicals were used by Fluka and Sigma International And devices with global origins. The material that used was: thiosemicarbazide(TSC), diacetylmonoxime(DAM),Sephadex G-25 and arginine as basic materials to the determination of arginase activity.This method given combines the enzymatic formation of urea with the sensitive method [10] . To measure the activity of arginase enzyme stimulating the last steep of urea cycle added DAM & TSC to give a solution with pink color.

The activity of arginase enzyme was measured after separated from serum by using separation column with 50cm length and 1.1cm filled with Sephadex G-25,then the activity of arginase enzyme calculated by the following equation:

$$(E_{\text{sample}_{530}} - E_{\text{blank}_{530}}) \times 38.9 = \mu \text{ moles} / \text{L serum} / \text{min.}$$

The factor = $3 \times 1000 / 0.6426 \times 120$ is derived from:

Sample volume = 0.5 mL. serum diluted 1:3.

conversion factor from mM to μM : 1000.

Slope of standard curve: $0.6426 \text{ E}^{530} / \text{mM}$.

Incubation time: 120 min

Serum Cholesterol , triglyceride , creatinine , uric acid , total protein and albumin were performed by commercially available kit (BIOLABO / France). Urea was performed by commercially available kit (Linear Chemicals / Spain). AST & ALT was performed by commercially available kit (RANDOX / UK).

2.3 Statistical analysis

In this study the results include mean \pm S.D and significant differences (P.Value) between groups that examined a available statical SPSS 17.0 significant differences was estimated as the p.value equal or less than 0.01.

3. Results

Table 1: mean \pm S.D of Arginase activity and other parameters for patients and control groups

Parameters	Control	Patients	P \leq
Mean \pm S.D	(n=35)	(n=65)	
Arginase activity ($\mu\text{mol/L/min}$)	0.331 \pm 0.0478	3.351 \pm 0.544	0.01
ALT (U/L)	17.08 \pm 0.565	51.34 \pm 1.21	0.01
AST (U/L)	15.662 \pm 0.508	40.28 \pm 1.45	0.01
Urea(mg/dl)	26.55 \pm 3.71	42.42 \pm 1.29	0.01
Creatinin(mg/dl)	0.929 \pm 0.248	1.665 \pm 0.127	0.01
Uric acid(mg/dl)	5.547 \pm 0.714	8.628 \pm 0.827	0.01
Cholesterol(mg/dl)	200.93 \pm 3.13	157.6 \pm 13.6	0.01
T.glyceride(mg/dl)	141.38 \pm 3.22	176.95 \pm 5.44	0.01
VLDL(mg/dl)	28.276 \pm 0.645	35.39 \pm 1.09	0.01
T.protine(g/dl)	6.65 \pm 0.365	4.662 \pm 0.415	0.01
Albumin(g/dl)	4.448 \pm 0.420	2.507 \pm 0.471	0.01
Globulin(g/dl)	2.203 \pm 0.539	2.028 \pm 0.616	0.05

Table 2: correlation coefficient between Arginase activity and biochemical parameters with typhoid fever.

Parameters	r value (patients)
Arg & ALT	0.272
Arg & AST	-0.343
Arg & Urea	-0.363
Arg & Creatinin	-0.769
Arg & Uric acid	0.938
Arg & Cholesterol	0.620
Arg & T.glycride	0.369
Arg & VLDL	0.369
Arg & T.Protine	-0.399
Arg & Albumin	-0.359
Arg & Globulin	0.161

4. Discussion

From table (1), the results show that there is a significant increase in the arginase activity for patients with typhoid fever compared to the healthy group. The increase in the levels of reactive oxygen species (ROS) in patients with typhoid fever is consider as the main reason to increase the secretion of the enzyme arginase [11]. One of the most affected cells by typhoid fever is hepatic cells due to hepatic inflammation, which is a major complicated reported for typhoid fever. [12,13] Typhoid fever is associated with hepatic hyperplasia and liver dysfunction [14] An increase in the level of liver enzymes is occurred due to a change in the function and structure of the liver. This change can cause necrosis of hepatic cells consequently, the enzyme discharge for the liver into the bloodstream [15] This significant effect on liver enzymes by the Salmonella infection of typhoid fever is related to several factors, including endotoxin, topical infections, or host-induced immune responses [16]. About 2-3 % of patients with typhoid fever has kidney complications, and renal damage may occur due to direct infection of kidney filtration units, leading to an increase in the level of Urea [17].

The increase in serum creatinine is most likely attributed to impaired glomerular filtration of urea and creatinine. The creatinine level also increased due to muscle injury. The high level of creatinine has been documented well in many diseases such as hepatitis, typhoid, urinary infections, kidney infections, and diabetes, which reveals the presence of acute renal failure [18]. Heavy exercise can also increase the level of creatinine in the blood, drugs furthermore can increase the level of creatinine in the blood by inhibition of creatinine secretion [19]. Uric acid commonly is the final result of the metabolism of purines, which can be taken as evidence for the oxidation process occurrence in addition to a therapeutic role as antioxidant [20]. The serum lipid level was also investigated in this research, one to the fact that cholesterol which is resulted from oxidation by free radicals will be attacked by active oxygen species (ROS) [21]. And the mechanism

responsible for the high level of T.G may be due to increased liver secretion of VLDL-C , a delayed in TG-rich lipoprotein purification , or increase in the levels of CFCs and free fatty acids [22] .VLDL contains large amounts of triglycerides formed in the liver by cortical hepatic cells, which act as transferees triglycerides, cholesterol ,and fat to the rest of the body [23]. The study [24] showed that oxidation in the body contributes to raise the level of VLDL.

The level of total proteins decreasing in this study can be related to the fact that it is an antioxidant outside the cellular and participates in many important reactions leading to stop the chain of reactions, such as fat staining, and hydroperoxides. It also contributes to hinder the reactions of free radicals in cellular membranes consequently, the membranes from crashing [25,26] The concentration of albumin in the serum is reduced by its consumption as antioxidant in the blood [27].

From table (2) it can be seen the relation of arginase activity with variables biochemical in patients with Typhoid fever as shown below

- 1- positive among the arginine activity and uric acid, triglycerides, VLDL, cholesterol I, ALT, and Globulin.
- 2- negative among the arginine activity and AST, urea, creatinine, total protein and albumine.

5. Conclusion

An investigated of typhoid fever case was successfully performed, studying its effect on the arginase activity. The main concluded points from this research were summarized as follow : the raising of arginase enzyme activity level ; increasing the level of ALT and AST liver enzymes , dropping of cholesterol level ,lift in triglycerides and VLDL level because of the lipid peroxidation , occurrence and moving of the level of vital indicators of renal function (Urea , creatinin and uric acid) .

6. Recommendations

The present study recommends the following:

1. To carry out more biotechnologies to isolate arginase enzyme such as electrical migration, ion exchange and other techniques to obtain high purity.
2. Isolation of other enzymes related to typhoid fever.
3. Study the effect of drugs on the biochemical variables in people with typhoid fever.

References

- [1]. Whitaker JA, Franco-Paredes C, Del Rio C, Edupuganti S. Rethinking typhoid fever vaccines: implications for travelers and people living in highly endemic areas. *Journal of travel medicine*. 2009 Jan 1;16(1):46-52.

- [2]. Hayat, A.S.; Shaikh, N. and Ahmed Shah S.I.; Typhoid fever: Evaluation of typhoid (IGM) in the early and rapid diagnosis of typhoid fever. *Professional Med J* 2011;18(2):259- 264.
- [3]. Crump, J.A. and Mintz, E.D.; Global trends in typhoid and paratyphoid fever. *Clin Infect Dis.* 2010; 50(2):241-246.
- [4]. Chalya, P.L.; Mabula, J.B.; Koy, M.; Kataraihya, J.B.; Jaka, H.; Mshana, S.E.; Mirambo, M.; Mchembo, M.D.; Giiti, G. and Gilyoma, J.M.; Typhoid intestinal perforation at a university teaching hospital in Northwestern Tanzania: A surgical experience of 104 cases in a resource-limited setting. *World Journal of Emergency Surgery* 2012;7:4.
- [5]. Zaki, S.A. and Karande, S.; Mu.-resistant typhoid fever: a review *Infect Dev Ctries* 2011;5(5):324-37.
- [6]. Yu HR, Sheen JM, Hwang KP, Kuo HC, Huang EY, Huang YC. Typhoid fever in southern Taiwan: a medical center experience. *Pediatrics & Neonatology.* 2008 Aug 1;49(4):116-20.
- [7]. Wongsawat, J.; Pancharoen, C. and Thisyakorn, U; Typhoid fever in children: experience in King Chulalongkorn Memorial Hospital. *J Med Assoc Thai* 2002;85(12):1247-1250.
- [8]. Nelson, D. L.; Cox, M. M. *Lehninger principles of biochemistry*, 6th Ed. W. H. Freeman & Company 2013; 704-706.
- [9]. Cederbaum SD, Yu H, Grody WW, Kern RM, Yoo P, Iyer RK. Arginases I and II: do their functions overlap?. *Molecular genetics and metabolism.* 2004 Apr 1;81:38-44.
- [10]. Coulombe JJ, Favreau L. A new simple semimicro method for colorimetric determination of urea. *Clinical chemistry.* 1963 Feb 1;9(1):102-8.
- [11]. Kuo L, Hein T. Vasomotor regulation of coronary microcirculation by oxidative stress: role of arginase. *Frontiers in immunology.* 2013 Aug 19;4:237.
- [12]. Abd-Alrazaq, F. S. & Ali, S. J. A study of the Biochemical and Haematological parameters in Patients with Typhoid Fever. *Diyala Journal For pure science*, 2017. Vol(3), No(4).
- [13]. Enemchukwu BN, Ibe CC, Udedi SC, Iroha A, Ubaoji KI, Ogundapo SS. Liver function assessment in malaria, typhoid and malaria-typhoid co-infection in Aba, Abia State, Nigeria. *Pakistan journal of biological sciences: PJBS.* 2014 Jun;17(6):860-3.
- [14]. Karoli R, Fatima J, Chandra A, Singh G. Salmonella hepatitis: an uncommon complication of a common disease. *Journal of family medicine and primary care.* 2012 Jul;1(2):160.
- [15]. Bolkent Ş, Saçan Ö, Karatuğ A, Yanardağ R. The effects of vitamin B6 on the liver of diabetic rats: A morphological and biochemical study. *IUFS Journal of Biology.* 2008;67(1):1-7.

- [16]. Ibrahim MM, Salihu A, Dugje FI. Typhoid Fever and Liver Enzyme Activity. *International Journal of Research*. 2015 Nov 18;2(11):656-62.
- [17]. Kang DH, Nakagawa T, Feng L, Watanabe S, Han L, Mazzali M, Truong L, Harris R, Johnson RJ. A role for uric acid in the progression of renal disease. *Journal of the American Society of Nephrology*. 2002 Dec 1;13(12):2888-97.
- [18]. Ndukaku OY, Emmanuel EU, Mercy EA, Caroline NO. Evaluation of the Serum Liver Enzymes Markers, Lipid Profile and Kidney Function Parameters in Typhoid Patients.
- [19]. College of Family Physicians of Canada. Elevated levels of serum creatinine. Guidelines for management and referral. *Canadian Society of Nephrology. Canadian Family Physician*. 2000 Mar 1;46(3):661-3.
- [20]. Berneis KK, Krauss RM. Metabolic origins and clinical significance of LDL heterogeneity. *Journal of lipid research*. 2002 Sep 1;43(9):1363-79.
- [21]. BAYIM BP, ADA FB, NZOEGWU PN. Status of oxidative stress in typhoid fever patients among fishers of calabar, cross river state, Nigeria. *World Journal of Life Sciences and Medical Research*. 2012 Jan 1;2(1):33.
- [22]. Chang YC, Chuang LM. The role of oxidative stress in the pathogenesis of type 2 diabetes: from molecular mechanism to clinical implication. *American journal of translational research*. 2010;2(3):316.
- [23]. Kanda E, Ai M, Okazaki M, Maeda Y, Sasaki S, Yoshida M. The association of very-low-density lipoprotein with ankle-brachial index in peritoneal dialysis patients with controlled serum low-density lipoprotein cholesterol level. *BMC nephrology*. 2013 Dec;14(1):212.
- [24]. Salem S.F., Badr M.O.T. & Neamat-allah A.N.F. Biochemical and pathological studies on the effect of levamisole and on Ehrlich ascites carcinoma-bearing mice. 2011;47,(1),89-95.
- [25]. Halliwell B, Gutteridge JM. *Free radicals in biology and medicine*. Oxford University Press, USA; 2015.
- [26]. Shimonov M, Pinchuk I, Bor A, Beigel I, Fainaru M, Rubin M, Lichtenberg D. Susceptibility of serum lipids to copper-induced peroxidation correlates with the level of high density lipoprotein cholesterol. *Lipids*. 1999 Mar 1;34(3):255-9.
- [27]. Ebisch IM, van Heerde WL, Thomas CM, van der Put N, Wong WY, Steegers-Theunissen RP. C677T methylenetetrahydrofolate reductase polymorphism interferes with the effects of folic acid and zinc sulfate on sperm concentration. *Fertility and sterility*. 2003 Nov 1;80(5):1190-4.