

## EXAMINATION OF SERUM OROTIC ACID LEVELS IN EXPERIMENTAL CIRRHOsis

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### DeneySEL Sirozda Serum Orotik Asit Düzeylerinin İncelenmesi

#### Summary

Due to hepatocellular damage, NH<sub>3</sub> level is increased and levels of urea cycle enzymes are reduced. NH<sub>3</sub> causes increase in the synthesis of mitochondrial carbamoyl phosphate. After diffusing into cytosole, carbamoyl phosphate is directed to pyrimidine synthesis by cytosolic carbamoyl phosphate synthase and increase in orotic acid production is observed. Objective of the present study was to evaluate orotic acid levels in experimental cirrhosis induced by CCl<sub>4</sub>. Total of 36 rats (control: 17, experimental group: 19) were used in the study. Experimental cirrhosis was induced by CCl<sub>4</sub>. Total protein, albumin, AST, ALT, ALP and LDH levels in serum were determined by an autoanalyzer whereas orotic acid levels were determined with HPLC. There was no significant changes in total protein levels whereas albumin levels were reduced in both group of rats. AST, ALT, ALP, LDH and orotic acid levels were found to be significantly increased in cirrhotic rats compared to control ones (p<0.005). There was a statistically significant increase in orotic acid levels in rats with experimental cirrhosis. Therefore, orotic acid level, in addition to other liver function parameters such as AST, ALT, ALP, LDH may be routinely determined so that more useful and meaningful results can be obtained about pathogenesis of liver diseases especially cirrhosis.

**Key Words:** Orotic acid, cirrhosis, HPLC

#### Özet

Hepatosellüler hasara bağlı olarak NH<sub>3</sub> düzeyi yükselir ve üre döngüsü enzimlerinde azalma meydana gelir. Yükselen NH<sub>3</sub> düzeyi mitokondriyal karbamoil fosfat sentezinin artışına neden olur. Sitozole diffüz olduktan sonra karbamoil fosfat, sitozolik karbamoil fosfat sentetaz tarafından pirimidin sentezine yönlendirilir ve orotik asit üretiminde artış gözlenir. Bu çalışmanın amacı CCl<sub>4</sub> ile oluşturulan deneysel sirozda orotik asit düzeylerini değerlendirmektir. Çalışmada toplam 36 sıçan (kontrol= 17, deney grubu= 19) kullanıldı. Deneysel siroz CCl<sub>4</sub> ile oluşturuldu. Serum total protein, albumin, AST, ALT, ALP, ve LDH düzeyleri otoanalizörle tayin edilirken, orotik asit düzeylerinin tayininde HPLC kullanıldı. Her iki grupta total protein düzeylerinde anlamlı bir değişiklik yok iken, albumin düzeyleri azaldı. Kontrol ile karşılaştırıldığında, sirotik sıçanların AST, ALT, ALP, LDH ve orotik asit düzeylerinin anlamlı derecede arttığı görüldü (p<0.005). Deneysel siroz oluşturulan sıçanların orotik asit düzeylerinde istatistiki olarak anlamlı bir artış vardır. Bu nedenle, AST, ALT, ALP ve LDH gibi karaciğer fonksiyon parametrelerine ek olarak orotik asit düzeylerinin de rutin olarak saptanması ile, karaciğer hastalıklarının, özellikle de sirozun patogenezi hakkında daha faydalı ve anlamlı sonuçlar elde edilebilir.

**Anahtar Kelimeler:** Orotik asid, siroz, HPLC

#### Introduction

Excessive formation of fibrous tissue in liver destroys paranchymal cells and occupies surroundings of blood vessels and hence creates a resistance against portal blood circulation which is known to be liver cirrhosis. Although alcohol is the

main reason for generation of cirrhosis, chemical compounds such as carbon tetrachloride (CCl<sub>4</sub>) or viral agents such as infectious hepatitis can also cause cirrhosis (1-3). After taken into the body, CCl<sub>4</sub> is dispersed via absorption into all organs and tissues.

Symptoms related with fatty liver and damage to hepatocytes are observed after a few days from absorption of  $\text{CCl}_4$  (4). Due to hepatocellular irregularitis in  $\text{CCl}_4$ -induced experimental cirrhosis, protein synthesis in liver is disrupted and dysfunction of detoxification (5), increased  $\text{NH}_3$  concentration (6) are observed. In addition, reduced synthesis of arginin results in decrease in urea cycle enzymes which finally cause lowered urea synthesis and excretion of  $\text{NH}_3$  (7). Significantly increased levels of hepatic enzymes give important clues about pathogenesis of cirrhosis (8).

Orotic acid is an intermediate product of pyrimidine biosynthesis metabolic pathway. Pyrimidine synthesis is initiated with a reaction where carbamoyl phosphate is synthesized from  $\text{CO}_2$  and glutamine which is the source for nitrogen in the cytosole. Reaction is catalyzed by cytosolic carbamoyl phosphate synthase II which is different than mitochondrial one (9). Orotic acid is synthesized as important key intermediate product of pyrimidine biosynthesis pathway which is composed of six reactions (9, 10).

In present study we aimed at examining serum orotic acid levels in rats with  $\text{CCl}_4$ -induced experimental cirrhosis. Whether pyrimidine synthesis pathway is affected by cirrhosis was also evaluated.

### Materials and Methods

Total of 36 male Wistar albino (150-200 g) rats purchased from Firat (Euphrates) University Biomedical Unit were used in the present study. All animals received humane care in compliance with the guidelines of Firat University Research Council's criteria. Animals were housed four animals per cage where they had *ad libitum* rat chow and water in an air-conditioned room with 12 hr light/dark cycle and controlled temperature (20-25°C). Control group was composed of 17 rats whereas 19 rats were used for induction of cirrhosis.  $\text{CCl}_4$  (Merck, Darmstad, Germany) was used as a selective hepatotoxin for development of cirrhosis.  $\text{CCl}_4$  mixed with olive oil (3/4, v/v) was injected s.c. twice a week for six weeks at 0.15 mL/100 g body weight. Animals in control group received only olive oil via s.c. injection twice a week for 6 weeks (11-13). One of the  $\text{CCl}_4$  received animals fasted overnight was decapitated and its liver was examined histopathologically and development of cirrhosis was detected. Then, all

animals were sacrificed by decapitation, blood and liver samples were collected. Sera from blood were separated and analyzed immediately. Livers from all animals were examined by certified histopathologists. Serum orotic acid levels were determined by HPLC (Cecil 1100 series, England) according to Banditt (14). Levels of biochemical parameters (total protein, albumin, AST, ALT, ALP and LDH) were determined by using Olympus AU 600 autoanalyzer (Nevada, USA) with commercially available kits (Randox Laboratories Ltd., Antrim, UK).

Results were expressed as mean  $\pm$  standart deviation (SD). Student's *t* test was used for statistical analysis. The 5% level of significance was used to evaluate differences.

### Results

Results of biochemical parameters (total protein, albumin, AST, ALT, ALP, LDH) of control and experimental group are summarized in Table 1. As a result of generation of cirrhosis, albumin levels showed a significant reduction whereas AST, ALP, LDH and especially ALT levels increased significantly ( $p < 0.005$ ) indicating liver damage (Table 1). Slight reduction in total protein levels in  $\text{CCl}_4$ -treated group compared to controls was not statistically significant ( $p > 0.05$ ).

Orotic acid levels were determined to be increased in experimentally induced cirrhosis ( $86.2 \pm 12.4 \mu\text{g/mL}$ ) compared to control group ( $13.3 \pm 2.0 \mu\text{g/mL}$ ). Increased orotic acid levels were greater than six times and so statistically significantly higher than control group (Figure 1).

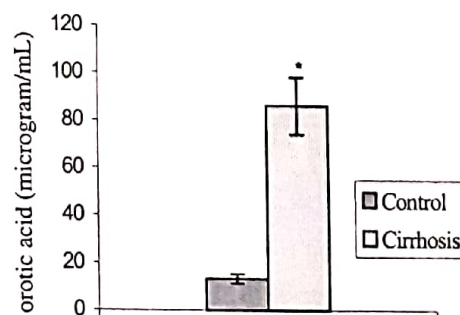


Figure 1. Serum orotic acid levels (\* $p < 0.005$ )

**Table 1.** Serum orotic acid and liver function tests results. (AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, LDH: Lactate dehydrogenase)

	Control (n:17)	Cirrhosis (n:19)
Total protein (g/dL)	7.26±0.28	7.07±0.37
Albumin (g/dL)	3.78±0.24	3.08±0.35*
AST (U/L)	179.0±27.60	891.0±151.1*
ALT (U/L)	57.8±11.0	590.53±121.17*
ALP (U/L)	155.8±45.5	411.16±136.12*
LDH (U/L)	3650.0±1004	4125.2±1231
Orotic acid (µg/mL)	13.3±2.0	86.2±12.4*

\*p&lt;0.005: Compared to control.

### Discussion

Glycine, alanine, serine, methionine, glutamate, ornithine, phenylalanine, tyrosine, histidine and proline levels were reported to be elevated at meaningful levels whereas isoleucine, arginine, leucine, aspartate, tryptophan and valine levels were decreased in cirrhotic rat livers (15,16). Levels of threonine, taurin, glutamine, lysine and citrulline were reported to be increased (15). Cirrhosis causes an increase in NH<sub>3</sub> levels in circulation and reduces ornithine transcarbamoylase activity (17,18). As a result of increased NH<sub>3</sub> concentration, synthesis of glutamine is increased (4). Similarly, liver arginase activity levels are decreased in cirrhotic rats (17). Increase in ornithine and lysin levels act as inhibitors of arginase (18).

All these events result in decrease of urea synthesis. Activities of urea cycle enzymes are lowered in several chronic liver diseases including cirrhosis (19-21). Metabolic alcoholosis formed due to decrease in urea cycle enzyme activities causes a reduction in excretion of bicarbonate as well as it stabilizes hepatic glutaminase (19). As a result of changes in glutaminase, NH<sub>3</sub> from glutamine and bicarbonate present in high concentration in the

environment are converted to carbamoyl phosphate by carbamoyl-phosphate synthase. Similarly, increased glycine levels due to liver cirrhosis also increases synthesis of mitochondrial carbamoyl phosphate levels (22). Carbamoyl phosphate whose concentration is increased in mitochondria diffuses into cytosole (23) where it enters into reactions of pyrimidine synthesis by aspartate transcarbamylase after reacting with aspartate. With this pathway, levels of orotic acid which is an intermediate product of pyrimidine synthesis is increased. Increased concentration of orotic acid in circulation causes orotic aciduria in CCl<sub>4</sub>-induced cirrhosis in rat liver (6,24,25). It is interesting to note that fatty liver is formed and lipid levels are increased in rats which fed with orotic acid in their diets (18,23,26,27,28). In addition, liver cirrhosis reduces ornithine transcarbamoylase activity (6,23) whereas orotic acid found in normal rat show increased activity of this enzyme (6,26).

In conclusion, orotic acid levels, in addition to routine biochemical parameters such as AST, ALT, ALP, LDH may be determined to decide about pathogenesis and prognosis of cirrhosis.

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