

## Effect of Fluoride Exposure on Serum Glycoprotein Pattern and Sialic Acid Level in Rabbits

Gulay Ciftci • Sena Cenesiz • Gul Fatma Yarim •  
Ozlem Nisbet • Cevat Nisbet • Metin Cenesiz •  
Dilek Guvenc

Received: 20 October 2008 / Accepted: 27 October 2009 /

Published online: 11 November 2009

© Humana Press Inc. 2009

**Abstract** This study describes the effects of fluoride exposure on the protein profile, glycoprotein pattern, and total sialic acid concentration of serum in rabbits. For this aim; 20 healthy New Zealand rabbits were used. The rabbits were divided into two equal groups each with ten animals according to their weighing: control group and experimental group. The rabbits in control group were given drinking tap water containing 0.29 mg/l sodium fluoride and experimental group received the same tap water to which was added 40 mg/l sodium fluoride for 70 days. Blood samples were taken from each rabbit on day 70. Serum fluoride concentrations were measured by a fluoride-specific ion electrode in serum. The fluoride levels in the serum were found as 18.4 ( $\pm 1.58$ )  $\mu\text{g/L}$  in control and 301.3 ( $\pm 52.18$ )  $\mu\text{g/L}$  in fluoride exposed rabbits. The sialic acid levels were found as 69.2 ( $\pm 0.32$ ) mg/dL in control and 43.4 ( $\pm 0.13$ ) mg/dL in fluoride exposed group. The electrophoretic patterns of serum proteins, glycoproteins, and total sialic acid concentration were determined. Fifteen different protein fractions with molecular weights ranging from 22 to 249 kDa were displayed in the serum protein electrophoretic gel of both groups. The raw concentrations of the protein fractions decreased in fluoride exposed rabbits as compared with the control rabbits. The serum glycoprotein pattern revealed seven major protein bands from 47 to 167 kDa in experimental and control groups. The slight decrease of raw concentration of the protein bands in glycoprotein pattern of serum was observed in fluoride toxication comparing to control. The

---

G. Ciftci (✉) • S. Cenesiz • G. F. Yarim • C. Nisbet  
Department of Biochemistry, Faculty of Veterinary Medicine, University of Ondokuz Mayıs,  
55139 Samsun, Turkey  
e-mail: gciftci@omu.edu.tr

O. Nisbet  
Department of Surgery, Faculty of Veterinary Medicine, University of Ondokuz Mayıs,  
55139 Samsun, Turkey

M. Cenesiz  
Department of Physiology, Faculty of Veterinary Medicine, University of Ondokuz Mayıs,  
55139 Samsun, Turkey

D. Guvenc  
Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine,  
University of Ondokuz Mayıs, 55139 Samsun, Turkey

results suggest that serum TSA determination and serum protein electrophoresis can be used to evaluate prognosis of fluoride exposure as a supplementary laboratory test in combination with clinical and other laboratory findings of fluorosis.

**Keywords** Biochemical profile · Fluoride · Glycoprotein · Sialic acid

## Introduction

Although contradictory scientific reports exist [1], fluoride is considered as an essential element for body metabolism [2–5]. Fluoride toxication is noxious to the health of humans and animals and is irreversible; it could be prevented by appropriate and timely intervention through understanding the processes at biochemical and molecular levels. High concentration of fluoride causes disorders such as endemic fluorosis and industrial fluorosis. Endemic fluorosis is related to the high concentration of fluoride present in the drinking water [6], while industrial fluorosis is mainly due to air pollution of fluoride in the working environment [7]. Fluorosis causes harmful effects in several organs in humans and animals [8]. Pathological changes occur in liver, kidney, heart, muscle, gastro-intestinal tract, and skeletal system, as a result of fluorosis [9]. In fluoride toxication, changes have been reported in biochemical parameters, due to elevation of oxidative stress [10]. Fluoride toxication results from increasing fluoride ions, which lead to inhibition of glycoprotein synthesis [11]. Some of the glycoproteins in serum are  $\alpha$ -1 acid protein (orosomukoid),  $\alpha$ -antitrypsin, haptoglobulin, ceruloplasmine, fibrinogen, and transferrin [12–14]. Carbohydrates found in the structure of serum glycoproteins are hexose, hexosamine, fucose, and sialic acid [15]. Some of the glycoproteins are involved in normal biological calcification of bone and teeth. Because of the calcium-binding property of sialic acid, for determining the glycoprotein levels in health and disease, sialic acid levels have been used as a marker [16].

Sialic acid is an acute phase protein derived from neuraminic acid which forms terminal sugar of carbohydrates taking part in the glycoprotein structure [17]. Sialic acids play a role in cell-cell recognition, protein targeting, protease resistance, conformational stabilization, adhesion, and intracellular signaling events in biological systems [18, 19]. Sialic acid is present at high percentage in the structure of  $\alpha$ -1 acid proteins,  $\alpha$ -antitrypsin, haptoglobulin, ceruloplasmin, fibrinogen, and transferrine, having a central role in the biological system [20]. Increases of sialic acid concentration have been reported in cardiovascular diseases [21], cancer [22], diabetes [23], patients with chronic glomerulonephritis, and chronic renal failure [24]. The level of blood serum sialic acid in health and illness situations is evaluated as a marker for glycoprotein amount [16].

In this study, it was aimed to demonstrate the effects of fluoride exposure on the protein profile, glycoprotein pattern, and total sialic acid concentration of serum in rabbits because of having the effects of fluorosis to the sialic acid levels and being sialic acid levels in relation to the glycoprotein and protein metabolism.

## Material and Methods

### Animal Material

In this study, a total of 20, healthy, 6-month-old New Zealand rabbits weighing  $3.5 \pm 0.4$  kg were used. Initially, an ethical approval for the study was received from the

Laboratory Animals Local Ethical Committee of Ondokuz Mayıs University, Samsun, Turkey. The rabbits were purchased from the Center of Medicinal and Surgical Research, Ondokuz Mayıs University and were kept at the same center during the research period. Rabbits were kept in hygienic conditions and well-ventilated environment at  $24 \pm 2^\circ\text{C}$ , with 60% relative moisture. The rabbits were randomly divided into two different groups: control group with ten animals and experimental group also with ten animals. Both groups were fed a standard rabbit chow consisting 0.5 mg/L sodium fluoride ad libitum and 12 h light/darkness regime was followed for 70 days. The rabbits in control group were given drinking tap water containing 0.29 mg/L fluoride (report from the Tap Water Purification Institution, Samsun Municipality, Samsun). Experimental group received the same tap water to which was added 40 mg/L sodium fluoride (Sigma-Aldrich Corporation, Saint Louis Mo, USA, Cat. No: S1504) for 70 days ad libitum as described by Akdogan et al. [25]. Blood samples were collected from marginal ear veins of rabbits on day 70.

### Analysis of Fluoride in Serum

The fluoride ion concentration was measured using a fluoride-specific ion electrode (ORION 9609BN, Thermo Electron Corp, MA, USA) that was connected to a digital ion analyzer (ORION EA 940, Orion Research, Inc). The electrode was previously calibrated with four standard fluoride solutions of 0.19, 1.9, 19, and 190 mg/L, respectively. Before measurement, 1 mL of each serum sample solution was pipetted into a clean plastic test tube and 1 mL of TISAB II (total ionic strength adjustment buffer, Orion Research, Inc, Beverly, MA, USA) concentrate with 1,2-cyclohexylenedinitrotetraacetic acid (Thermo Orion, MA, USA) was added to each solution. The concentration (millivolt) of each solution was directly read out on the instrument display. Data concerning fluoride was recorded in milligram per liter.

### Biochemical Analyses

Blood samples were kept at ambient temperature for 30 min and centrifuged at  $+4^\circ\text{C}$ ,  $1,550 \times g$  for 10 min to obtain serum samples. These sera were stored at  $-80^\circ\text{C}$  until analyses. Serum concentrations of total protein, albumin, cholesterol, triglyceride, creatinine, ALT, AST (Sigma-Aldrich Chemie GmbH, Germany) were determined according to the manufacturer's instruction using auto-analyzer (Autolab, AMS Srl, Selective Access).

### Serum Protein Electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE; 10%) was performed according to Laemmli [26]. Serum samples were diluted with saline solution and mixed with sample buffer (0.062 M Tris-HCl, pH 6.8, 10% glycerol, 2% SDS, 5%  $\beta$ -mercaptoethanol, and 0.002% bromophenol blue), then heated for denaturation and run on SDS-PAGE. For SDS-PAGE, 20  $\mu\text{L}$  of samples were loaded on the stacking gel. A 200 V was applied until bromophenol blue came to the lowest side of the gel. At the end of electrophoresis, the proteins were stained with Blue Silver staining [27] or for glycoprotein as described below. Molecular mass standards (Sigma, S8445) were run in parallel in order to calculate the molecular weights of proteins by using Molecular Imaging Software (Kodak). Photographs of the gels were taken.

## Detection of Glycoproteins

Glycoproteins were determined on nitrocellulose filters using a commercially available digoxigenin glycan detection kit (Roche Molecular Biochemical). The manufacturer's recommendations were followed. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets was performed according to the method described by Towbin et al. [28]. Briefly, membranes were washed in phosphate buffer solution (50 mM potassium phosphate, 150 mM NaCl (pH 6.5)) and carbohydrates were oxidized with sodium metaperiodate. Oxidized carbohydrates were labeled with digoxigenin-conjugated hydrazide, and labeled proteins were visualized by alkaline phosphatase-conjugated antidigoxigenin antibodies, followed by a color reaction with Nitro Blue Tetrazolium-X-phosphate.

## Estimation of Serum Sialic Acid

Serum total sialic acid levels were estimated colorimetrically by tiobarbituric acid method [29]. Briefly, sera were incubated at 80°C for 1 h in 0.1 N sulphuric acid. The standard solution was prepared using sialic acid (*N*-acetylneuraminic acid, Sigma, USA) and a standard calibration curve was established using 25, 50, 75, and 100 µg/mL standard *N*-acetylneuraminic acid solutions. The optical density was read at 550 nm using a Spectra.

## Statistical Analysis

Pearson Correlation analysis was used to investigate the relationships between variations.

## Results

The serum biochemical parameters of control and fluoride-exposed group are presented in Table 1. The fluoride levels in the serum were found as 18.4 (±1.58) µg/L in control and 301.3 (±52.18) µg/L in fluoride exposed rabbits on day 70. In comparison to day 70 and 0, the increases in fluoride levels on serum were found as statistically important. The levels of serum albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, total protein, cholesterol, and triglyceride were 3.3 g/dL, 22.4 U/L, 33.2 U/L,

**Table 1** Serum Biochemical Profile of Control and Fluoride Exposed Rabbits

Serum parameters	Control group ( <i>n</i> =10)	Fluoride exposed group ( <i>n</i> =10)
Fluoride (µg/L)	18.4 (±1.58)	301.3 (±52.18)*
Albumin (g/dL)	3.3 (±0.1)	2.9 (±0.1)*
AST (U/L)	22.4 (±2.9)	30.0 (±2.5)
ALT (U/L)	33.2 (±2.6)	63.0 (±9.5)*
Creatinine (mg/dL)	0.6 (±0.04)	1.1 (±0.1)*
Total protein (g/dL)	7.2 (±0.0.2)	5.1 (±0.4)*
Cholesterol (mg/dL)	97.8 (±8.0)	64.4 (±9.8)*
Triglyceride (mg/dL)	96.0 (±3.4)	60.0 (±2.2)*

\**p*<0.05, statistically significant

0.6 mg/dL, 7.2 g/dL, 97.8 mg/dL, and 96.0 mg/dL, respectively, in control group; 2.9 g/dL, 30.0 U/L, 63 U/L, 1.1 mg/dL, 5.1 g/dL, 64.4 mg/dL, and 60.0 mg/dL, respectively, in fluoride-exposed group. Serum albumin, total protein, cholesterol, and triglyceride concentrations were significantly lower in experimental group compared with controls ( $p < 0.05$ ). In contrast, ALT and creatinine levels were increased ( $p < 0.05$ ) in fluoride-exposed rabbits relative to healthy rabbits. Although a slight increase was observed in AST activity in fluoride-exposed group, this increase was not statistically significant ( $p > 0.05$ ).

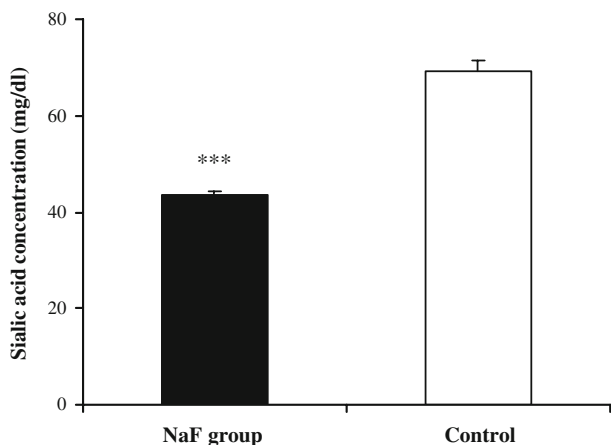
TSA concentration in fluoride-exposed group was significantly lower ( $p < 0.001$ ) compared with control rabbits (Fig. 1). Between fluoride and TSA levels in serum, correlation coefficient was found as  $r = -0.79$  ( $p < 0.01$ ). This negative correlation was indicated that increasing amount of fluoride level in serum was caused to decrease sialic acid level in serum.

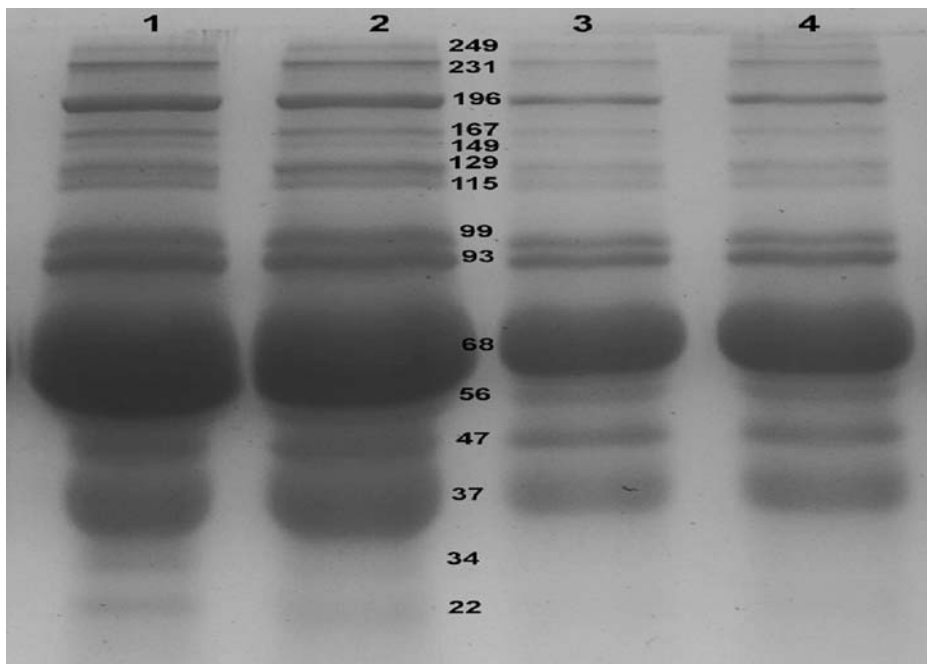
Fifteen different protein fractions with molecular weights ranging from 22 to 249 kDa were displayed in the serum protein electrophoretic gel of both groups (Fig. 2). The raw concentrations of the protein fractions decreased in fluoride exposed rabbits as compared with the control rabbits. The serum glycoprotein pattern revealed seven major protein bands including 167, 129, 115, 99, 93, 56, 47 kDa in experimental and control groups (Fig. 3). The slight decrease of raw concentration of the protein bands in glycoprotein pattern of serum was observed in fluoride toxication comparing to control.

## Discussion

Fluoride is known as toxic when taken excessive amounts ( $\geq 40$  mg/L sodium fluoride) and can cause various disorders in organism. Fluoride ions pass the intestinal barrier and are deposited in several organs and body through the blood system [30]. Liver, an essential and active organ, is most affected from fluoride toxication [31]. Fluoride is eliminated (50-80%) by kidney, therefore kidneys are also very sensitive to fluoride toxication [8]. It has been reported that some biochemical parameters change due to increased oxidative stress in fluoride toxication [10]. In our study, the total protein and albumin levels decreased in fluoride exposed rabbits compared to the control rabbits. These results were in agreement with previously published literature, in which some reduction in total protein and albumin

**Fig. 1** Serum total sialic acid concentrations (mean  $\pm$  SD) in fluoride exposed ( $n=10$ ) and control ( $n=10$ ) groups. \*\*\* $p < 0.001$

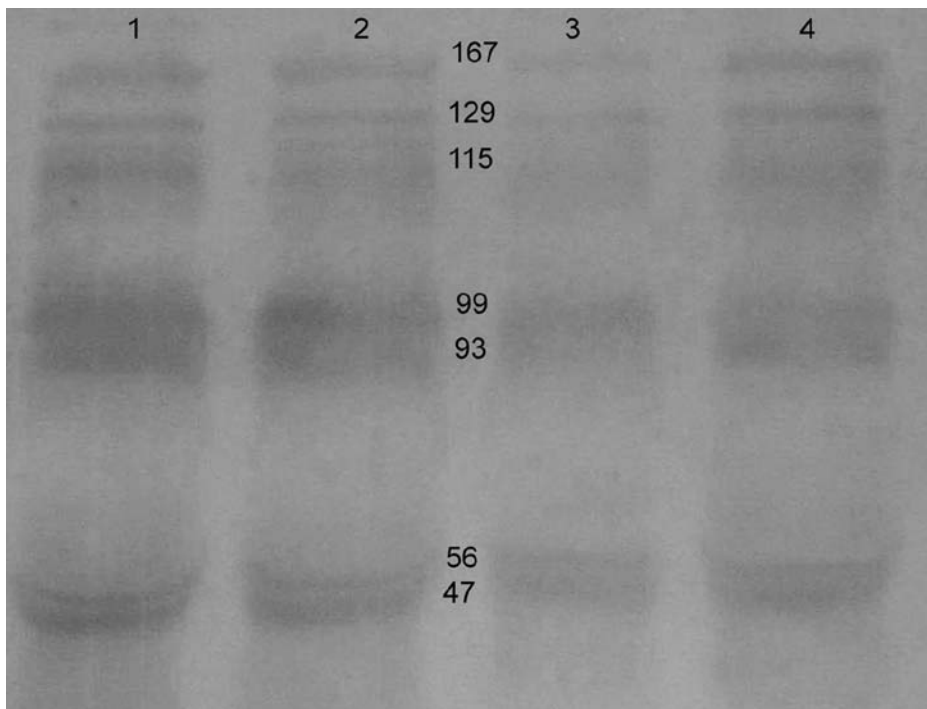




**Fig. 2** Electrophoretic pattern of serum proteins of healthy (lanes 1 and 2) and fluoride exposed group (lanes 3 and 4)

levels had been reported in fluoride toxication of rats [32], rabbit [33], weathers, and suckling pups [31].

Fluoride toxication affects protein synthesis by primary causing destruction of polypeptide chains [34] and weakness of amino-acid bindings in proteins [35]. It has been reported that excessive amount of fluoride cause a reduction of protein synthesis [31] and also metabolisms of cholesterol and triglyceride in liver are affected in fluoride-exposed group [36]. Comparing the control and experimental groups, even though the serum protein patterns were the same, a reduction in protein concentration in the fluoride-exposed group was determined. Wang et al. [37] have reported a statistically insignificant reduction in the levels of erythrocyte and cholesterol of the liver in rats with chronic fluorosis. However, in our study, a statistically significant reduction of serum cholesterol and triglyceride levels was determined. Hanen et al. [31] have reported a reduction in serum cholesterol and triglyceride levels in mice and suckling pups with chronic fluorosis. Furthermore, development of hypoglycemia was observed in chicks exposed to fluoride factors in atmosphere for 20 days [38]. In fluoride exposure, increases of AST and ALT activities, known as the sign of damage and dysfunction of liver occur [39]. Enzyme ALT revealed a statistically significant increase ( $p < 0.05$ ), while enzyme AST increased slightly but the latter was not statistically significant. Likewise, a statistically significant increase in ALT and AST enzymes have been reported in mice, suckling pups [31], and rat subjects [10, 40]. Kidney is one of the target organs for development of fluorosis toxication [41]. It has been reported that proximal curves of the tubular cells are damaged in acute fluorosis [42]. In this study, a statistically significant increase has been observed in serum creatinine levels. In parallel with our results, significant increases in creatinine levels have been previously



**Fig. 3** Electrophoretic pattern of serum glycoproteins of healthy (lanes 1 and 2) and fluoride exposed group (lanes 3 and 4)

reported in rabbit [25] and rat subjects [39]. This increase may be explained by the dysfunction of kidneys due to the damage which occurs in the fluoride-exposed group.

In exposure of fluoride, glycoprotein synthesis is inhibited by increased fluoride ions [11]. Most of the glycoproteins found in serum are  $\alpha$ -1 glycoproteins (orosomucoid),  $\alpha$ -antitrypsin, fibrinogen, haptoglobin, and transferrin [43, 44]. Carbohydrates found in serum glycoproteins are hexose, hexosamine, fucose, and sialic acid [45]. The serum protein and glycoprotein concentration were significantly low in the animals from the experimental group in comparison with the control. As the same of our study, it has been found a reduction in the amount of glycoproteins while an increase in the amount of fluoride in human serum [11]. This was explained by the inhibition of glycoprotein synthesis because of the increased amount of fluoride ions. In the present study, findings revealed a decrease in levels of sialic acid in serum with increasing water fluoride concentrations. Similarly, reduction in the amount of protein-bound sialic acid and glycoprotein was reported in rabbits with exposure of fluoride and it has been stated that decreased circulatory sialic acid levels with increased serum fluoride in rabbits [16]. Following fluorosis, some alterations can occur in calcium metabolism and various soft tissues can be calcified [36]. Because of having some evidences to indicate sialic acid in a relation to glycoproteins [46], the decreasing levels of the sialic acid in circulation in our study may be dependent on deposition of glycoproteins in the tissues by calcium bridges. Also; the reduction of sialic acid level may be due to the decrease in the biosynthesis or disruption of protein biosynthesis.

In conclusion, significant changes were observed in sialic acid level and patterns of serum protein and glycoprotein in fluoride exposed rabbits. The results suggest that, apart



from the measurement of fluoride in urine or serum as described by Usuda et al. [42, 47], serum protein and glycoprotein electrophoresis and sialic acid measurement can be used in the evaluation of fluoride exposure and this evaluation could expose the damages of the organs and protein metabolisms. However, further studies are needed to precisely assess the role of exposure of fluoride in specific protein profile and evaluate the meaning and usefulness of the protein electrophoresis during its course.

## References

1. Committee on Dietary Allowances (1989) Fluoride. In: Recommended dietary allowances, 10th edn. National Academy Press, Washington, DC. pp 235
2. Collier DR (1980) Fluorine: an essential element for good dental health. *J Public Health Dent* 40: 296–300
3. Robinson C, Kirkham J (1990) The effect of fluoride on the developing mineralized tissues. *J Dent Res* 69:685–691
4. Yamaguchi M (2007) Fluoride and bone metabolism. *Clin Calcium* 17:217–223
5. Zlotkin SH, Atkinson S, Lockitch G (1980) Fluorine: an essential element for good dental health. *J Public Health Dent* 40:296–300
6. Li J, Cao S (1994) Recent studies on endemic fluorosis in China. *Fluoride* 27:125–128
7. Czervinski E, Nowak J, Dabrowska D et al (1988) Bone and joint pathology in fluoride-exposed workers. *Arch Environ Health* 43:340–343
8. Xiong X, Liu J, He W et al (2007) Dose–effect relationship between drinking water fluoride levels and damage to liver and kidney functions in children. *Environ Res* 103:112–116
9. Cetin N, Bilgili A, Eraslan G et al (2004) Effect of fluoride application on some blood parameters in rabbits. *J Health Sci* 13:46–50
10. Zhang M, Wang A, He W et al (2007) Effects of fluoride on the expression of NCAM, oxidative stress, and apoptosis in primary cultured hippocampal neurons. *Toxicol* 236:208–216
11. Sushella AK, Sharma YD, Rajalakshmi MKJ et al (1981) Chemical profile of human serum in fluoride toxicity and fluorosis. I. Total proteinbound carbohydrates, seromucoid and fluoride levels. *Fluoride* 14:150–154
12. Flahaut C, Michalski JC, Danel T et al (2003) The effects of ethanol on the glycosylation of human transferrin. *Glycobiol* 13:191–198
13. Taniuchi K, Chifu K, Hayashi N et al (1981) A new enzymatic method for the determination of sialic acid in serum and its application for a marker of acute phase reactants. *J Med Sci* 27:91–102
14. Wang JS, Tsutsumi M, Takada A (1992) Microheterogeneity of serum glycoproteins in alcoholic liver disease. *Nippon Shokakibyo Gakkai Zasshi* 89:1407–1414
15. Wiese TJ, Dunlap JA, Yorek MA (1997) Effect of L-fucose and D-glucose concentration on L-fucoprotein metabolism in human Hep G2 cell and changes in fucosyltransferase and  $\alpha$ -L fucosidase activity in liver of diabetic rats. *Biochim Biophys Acta* 17:61–72
16. Sharma YD (1983) Serum sialic acid and ceruloplasmin levels in experimental fluorosis. *Toxicol Lett* 15: 1–5
17. Chrostek L, Cylwik B, Korcz W et al (2007) Serum free sialic acid as a marker of alcohol abuse. *Alcoholism* 31:588–592
18. Varki NM, Varki A (2007) Diversity in cell surface sialic acid presentation: Implications for biology and disease. *Lab Invest* 87:851–857
19. Schauer R, Kelm S, Reuter G et al (1995) Biochemistry and role of sialic acids. In: Rosenberg A (ed) *Biology of the sialic acids*. Plenum Publishing Corp, New York
20. Flahaut C, Michalski JC, Danel T et al (2003) The effects of ethanol on the glycosylation of human transferrin. *Glycobiol* 13:191–198
21. Serdar Z, Yesilbursa D, Dirican M et al (2007) Sialic acid and oxidizability of lipid and proteins and antioxidant status in patients with coronary artery disease. *Cell Biochem Funct* 25:655–664
22. Kokoglu E, Sonmez H, Uslu E et al (1992) Sialic acid levels in various types of cancer. *Cancer Biochem Biophys* 13:57–64
23. Crook MA, Pickup JC, Lumb PJ et al (2001) EURODIAB IDDM Complications Study Group. Relationship between plasma sialic acid concentration and microvascular and macrovascular complications in type 1 diabetes: The EURODIAB Complications Study. *Diabetes Care* 24:316–322



24. Jaaskelainen IP (1999) Occurrence of sialic acids in healthy humans and different disorders. *Eur J Clin Invest* 29:413–425
25. Akdogan M, Bilgili A, Kara EZ et al (2002) The structural and biochemical changes of kidney tissue on fluorosis in rabbits. *Turk J Vet Anim Sci* 26:71–77
26. Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227:680–685
27. Candiano G, Bruschi M, Musante L et al (2004) Blue silver: a very sensitive colloidal Coomassie G-250 staining for proteome analysis. *Electrophoresis* 25:1327–1333
28. Towbin H, Staehelin T, Gordon J (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA* 76:4350–4354
29. Warren L (1959) The thiobarbituric acid assay of sialic acids. *J Biol Chem* 234:1971–1975
30. Mullenix PJ, Denbesten PK, Schunior A (1995) Neurotoxicity of sodium fluoride in rats. *Neurotoxicol Teratol* 17:169–177
31. Bouaziz H, Ketata S, Jammoussi K et al (2006) Effects of sodium fluoride on hepatic toxicity in adult mice and their suckling pups. *Pest Biochem Physiol* 86:124–130
32. Verma RJ, Sherlin DM (2002) Sodium fluoride-induced hypoproteinemia and hypoglycemia in parental and F1-generation rats and amelioration by vitamins. *Food Chem Toxicol* 4:1781–1788
33. Shashi SP, Thapar P, Singh JP (1987) Effect of fluoride administration on organs of gastrointestinal tract—an experimental study on rabbits-effect on tissue protein. *Fluoride* 20:183–188
34. Macuch P, Balazoa G, Bartozoa L et al (1963) Hygienic analysis of the influence of noxious factors on the environment and state of health of the people in the vicinity of aluminium plants. *J Hyg Epidemiol* 7:389–400
35. Helgeland K (1976) Effect of fluoride on protein and collagen biosynthesis in rabbit dental pulp in vitro. *Scand J Dent Res* 84:276–285
36. Singh JP, Shashi A, Thapar SP (1985) Effect of fluoride on total lipid, cholesterol and triglyceride levels in liver of albino rabbits. *Fluoride* 18:146–149
37. Wang Y, Xiao K, Liu J et al (2000) Effect of airborne fluoride on some hematological parameters of chick. *Toxicol* 146:161–169
38. Pillai KS, Mane UH (1985) Effect of airborne fluoride on some haematological parameters of chick. *Bull Environ Contam Toxicol* 35:510–516
39. Zabulyte D, Uleckiene S, Kalibatas J (2006) Experimental studies on effect of sodium fluoride and nitrate on biochemical parameters in rats. *Bull Vet Inst Pulawy* 51:79–82
40. Grucka-Mamczar E, Machoy Z, Tarnawski R et al (1997) Influence of long-term sodium fluoride administration on selected parameters of rat blood serum and liver function. *Fluoride* 3:157–164
41. Pessan JP, Pin ML, Martinhon CCR et al (2005) Usefulness of the assessment of urinary enzyme leakage in monitoring acute fluoride nephrotoxicity. *Caries Res* 39:363–370
42. Usuda K, Kono K, Dote T et al (1999) Usefulness of the assessment of urinary enzyme leakage in monitoring acute fluoride nephrotoxicity. *Arch Toxicol* 73:346–351
43. Flahaut C, Michalski JC, Danel T et al (2002) The effects of ethanol on the glycosylation of human transferrin. *Glycobiology* 13:191–198
44. Chrostek L, Cylwik B, Korcz W et al (2007) Serum free sialic acid as a marker of alcohol abuse. *Alcohol Clin Exp Res* 31:996–1001
45. George J, Chandrakasan G (1996) Glycoprotein metabolism in dimethylnitrosamine induced hepatic fibrosis in rats. *Int J Biochem Cell Biol* 28:353–361
46. Morell AG, Gregoriadis G, Scheinberg IH (1971) The role of sialic acid in determining the survival of glycoproteins in the circulation. *J Biol Chem* 246:1461–1467
47. Usuda K, Kono K, Shimbo Y et al (2007) Urinary fluoride reference values determined by a fluoride ion selective electrode. *Biol Trace Elem Res* 119:27–34