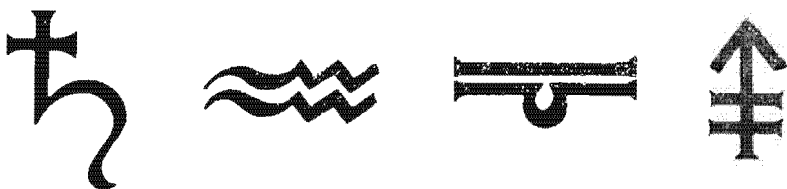


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**Excessive Ingestion of Fluoride and
the Significance of Sialic Acid:
Glycosaminoglycans in the Serum of
Rabbit and Human Subjects**

MOHAN JHA
A. K. SUSHEELA*

Fluorosis Research Laboratory
Department of Anatomy
All India Institute of Medical Sciences
New Delhi 110029, India

NEELAM KRISHNA
K. RAJYALAKSHMI

Institute of Preventive Medicine
Hyderabad (Andhra Pradesh), India

K. VENKIAH

Department of Biochemistry
Gandhi Medical College
Hyderabad (Andhra Pradesh), India

ABSTRACT

The levels of sialic acid and glycosaminoglycans were explored in the sera of rabbit and human subjects who ingested fluoride and had clinical manifestation of fluorosis. Changes observed in the

*To whom requests for reprints and all correspondence should be addressed.

level of these chemical constituents in sera possibly reflect changes occurring in calcified and noncalcified tissues due to fluoride intoxication. The ratio of sialic acid content vs glycosaminoglycans revealed there was a 50% reduction in rabbit and human sera. The test is recommended for evaluating the prognosis of fluoride poisoning/fluorosis.

Glycosaminoglycans and glycoproteins form an integral part of the organic matrix of bone which is constituted predominantly of collagen fibers. A detailed analysis of glycosaminoglycans (GAG) and their status due to fluoride ingestion in cancellous and cortical bone of rabbit has been reported [1]. The authors also investigated one of the pathological lesions that occurs in the trabeculae of cancellous bone and have evaluated the extent of accumulation of GAG in these sites by quantitative and qualitative methods [2, 3].

Besides glycosaminoglycans, the glycoprotein profile of sialic acid (N-acetyl-neuraminic acid) in cancellous and cortical bone has also been investigated. According to Susheela and Jha [4], sialic acid is present in the same amount in both cancellous and cortical bone. However, the response of sialic acid content to fluoride ingestion differs significantly between cancellous and cortical bones. The sialic acid content with fluoride ingestion is significantly enhanced in cancellous bone and decreased in cortical bone. The changes observed in glycosaminoglycans and glycoprotein content in tissues of rabbit have led the investigators to explore the status of these constituents in the sera of both rabbits and human subjects afflicted with fluorosis. The present report provides data on glycosaminoglycans and sialic acid in human and rabbit sera and elucidates the significance of the study.

MATERIALS AND METHODS

Human Subjects Afflicted with Fluorosis

Nine human subjects (age ranging from 17 to 30) living in endemic areas of Andhra Pradesh (South India) where the potable water has a fluoride content ranging from 8.1 to 8.6 ppm (permissible limits of fluoride is 1 to 1.5 ppm) and ailing from skeletal and dental fluorosis for a period of 5 to 15 yr were chosen for the present investigation. The human subjects have skeletal fluorosis of mild, moderate, and severe forms. The individuals also have dental fluorosis from Grades I to III (Table 1). The clinical manifestations of the mild, moderate, and severe forms of skeletal fluorosis and Grades I, II, and III of dental fluorosis were as follows.

TABLE 1. Information about the Patients

No.	Age (yr)	Sex	Duration of illness (yr)	Fluoride content in water source (ppm)	Fluorosis grade		Clinical manifestations
					Skeletal	Dental	
1	27	M	13	8.1	Moderate	I	Backache, difficulty in walking for long distance, limping
2	30	M	12	8.1	Mild	II	Backache, difficulty in walking for long distance
3	22	M	10	8.1	Mild	II	Backache, difficulty in bending, joint pain
4	30	M	15	8.2	Moderate	II	Backache, difficulty in walking, joint pain
5	20	M	8	8.1	Moderate	II	Difficulty in walking, joint pain
6	30	M	5	8.6	Moderate	III	Backache, joint pain
7	17	M	12	8.2	Severe	II	Backache, difficulty in walking, joint pain, criss-cross movements while walking
8	18	M	12	8.1	Severe	II	Criss-cross movements while walking, joint pain
9	25	F	10	8.1	Severe	III	Backache, cervical stiffness, stiffness of joints, and joint pain

Skeletal Fluorosis

Mild form: The subjects were asymptomatic but radiographs revealed increased bone density.

Moderate form: The subjects were symptomatic with aches and pain in joints and vertebral column and had difficulty in walking.

Severe form: The subjects were symptomatic with marked limitations in the movements of the spine and joints with cervical stiffness and crippling deformities.

Dental Fluorosis

Grade I: The teeth had white opacities and faint yellow lines.

Grade II: The teeth had brown stain.

Grade III: The teeth had brown lines with pitting and chipped off edges.

Animal Model

Adult healthy rabbits received daily 10 mg sodium fluoride/kg body weight by the intragastric route for a period of 8 months.

Estimation of Serum Fluoride Content

Sera obtained from human subjects and rabbits were used for fluoride estimation. Serum fluoride was estimated by the method of Hall et al. [5] using a fluoride ion specific electrode in a PHM 84 Research pH Meter (radiometer). The fluoride content of sera was expressed as parts per million (ppm).

Estimation of Sialic Acid Content

Sialic acid content in human and rabbit sera was estimated according to the method of Winzler [6]. To 0.2 mL of sera, 4.8 mL of 5% trichloroacetic acid was added and placed in a boiling water bath for 15 min. The samples were centrifuged at 2000 rpm for 10 min. Aliquots of the supernatant were used for color development with diphenylamine (DPA) reagent. The diphenylamine reagent was prepared by dissolving 1 g of diphenylamine in glacial acetic and concentrated sulfuric mixed in the ratio of 9:1. Correction for nonspecific color development was applied. Optical density measurements were made at 530 nm using a Zeiss PMQ II Spectrophotometer. Results were expressed as mg/100 mL sera.

Estimation of Serum Glycosaminoglycans

Glycosaminoglycan content of human and rabbit sera were estimated according to the method of Gold [7]. To a known volume of serum (0.2 mL), 1.2 mL of alcian blue reagent was added, and optical density measurements were made after 10 min at 488 nm in a Zeiss PMQ II Spectrophotometer. The alcian blue reagent was prepared in 0.5 M sodium acetate to produce of final dye concentration 1.4 mg/mL. Chondroitin sulfate was used as the standard. The results were expressed as mg/100 mL sera.

RESULTS

The data obtained for fluoride content, sialic acid content, and glycosaminoglycan levels of sera of rabbit and human are reported in Table 2. The circulating levels of fluoride in rabbit and human sera are elevated after fluoride ingestion. The sialic acid content of rabbit and human sera decreased after excessive ingestion of fluoride. Enhanced levels of glycosaminoglycans occur in rabbit and human sera after fluoride intoxication. More than 50% reduction in the ratio of sialic acid:glycosaminoglycans was observed in rabbit sera. A 50% reduction of this ratio in human sera was seen.

DISCUSSION

Enhanced fluoride content in blood sera has been reported in animal and humans afflicted with fluorosis [1, 8]. Our data on fluoride content in the present communication are in agreement with the earlier data.

It is also possible to offer an explanation for reduced sialic acid content in serum after fluoride ingestion, as sialic acid is known to bind with calcium ions [9, 10] and is deposited in cancellous bone [4, 11], lowering its levels in circulation. Reduced sialic acid content in rabbit sera is consistent with our previous report [4].

Although glycosaminoglycans are known to be raised in serum in osteoarthritis [12], changes in glycosaminoglycans content in cancellous bone is highly specific due to fluoride ingestion [3]. The exact loci in trabecular bone having excessive glycosaminoglycans content has also been identified [2]. Our recent studies on ³⁵S-sulfate uptake by calcified and noncalcified tissues have also revealed that proteoglycan synthesis is enhanced in kidney, liver, skin, cancellous, cortical bone, and tooth during fluoride ingestion [13]. The enhanced glycosaminoglycans content in various tissues is reflected in circulating levels of glycosaminoglycans as well.

TABLE 2. Serum Fluoride, Sialic Acid (SA), and Glycosaminoglycans (GAG) Content of Normal and Fluorosed Human Subjects and Rabbits^a

	Fluoride (ppm)	Sialic acid (mg/100 mL)	Glycosaminoglycans (mg/100 mL)	SA/GAG
Rabbit:				
Normal (5)	0.07 ± 0.02	61.97 ± 1.89	6.08 ± 1.21	10.19
Fluorosed (5)	0.45 ± 0.06**	42.71 ± 4.78*	13.27 ± 1.57*	3.22
Human:				
Normal (5)	0.02 ± 0.01	61.09 ± 2.72	9.45 ± 0.53	6.46
Fluorosed (5)	0.19 ± 0.15*	45.39 ± 2.68**	12.20 ± 1.51**	3.72

^aNumber in parenthesis indicates the number of samples.

*p < 0.05.

**P < 0.01.

A 50% reduction in the ratio of sialic acid verses glycosaminoglycans has been observed both for animal models and human subjects intoxicated with fluoride. It is therefore proposed that the ratio of sialic acid/glycosaminoglycans be considered as a prognostic test for fluorosis/fluoride intoxication. Considering the merits of the test (simplicity of method, reproducibility of results, and sensitivity of the test), it is strongly recommended for clinical diagnosis. Efforts are being made to evaluate the validity of the test for the prognosis of mild, moderate, and severe forms of fluorosis.

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