

Circulating levels of sialic acid and glycosaminoglycans: a diagnostic test for ankylosing spondylitis

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SUMMARY The circulating levels of sialic acid (*N*-acetylneuraminic acid) and glycosaminoglycans (GAGs) were measured in 69 patients with spinal disorders of orthopaedic interest (ankylosing spondylitis 17, osteofluorosis 6, idiopathic backache 10, osteoarthritis 16, osteoporosis 20). The serum GAG levels showed no statistically significant change from control values in the five disorders investigated in the present study. Although osteoporosis and osteoarthritis showed a decrease in serum sialic acid (SA) levels, the mean ratio (SA/GAG) demonstrated no change from control values. Idiopathic backache showed no difference in any of the parameters studied when compared with control values. Ankylosing spondylitis and osteofluorosis had a remarkable similarity in their clinical and radiological features, but a divergent mean value of ratio was noted. The mean ratio of both the conditions also showed a statistically significant difference from the control value. This suggests that the SA/GAG ratio can be used as a diagnostic test in ankylosing spondylitis.

Key words: osteofluorosis, idiopathic backache, osteoarthritis, osteoporosis.

Ankylosing spondylitis (AS) is a chronic inflammatory disease affecting mainly the spine and sacroiliac joints, but in some cases arthritic changes occur also in the peripheral joints.¹ Sacroiliitis has been traditionally regarded as the hallmark of AS. This radiological feature is also seen in other orthopaedic conditions, however.² Erythrocyte sedimentation rate (ESR), acute phase proteins such as C reactive protein (CRP), and immunoglobulin (IgG, IgA) concentrations are some of the important laboratory markers of AS. These markers are, however, unreliable diagnostically owing to lack of specificity and poor correlation with disease activity.^{3,4} A very high prevalence of HLA-B27 in patients with AS has been reported by various authors.^{5,6} All B27 positive individuals do not develop AS, however, and the B27 negative and positive patients of AS demonstrate identical skeletal manifestations.⁷ The asso-

ciation of the B27 antigen with other seronegative arthropathies, such as Reiter's disease⁸ and reactive arthritis after gastrointestinal infection,⁹ further limits the diagnostic relevance of HLA-B27 in AS. As there is no specific diagnostic test for AS, misdiagnosis is not uncommon, especially if the disease begins in the peripheral joints or with iritis or aortitis.¹⁰

Proteoglycans and sialoprotein are the two significant components in mineralised bone matrix.¹¹ These matrix components are known to be important for the maintenance of bone structure and calcification. It is thus to be expected that bone disorders would bring about a change in their contents.

Bone biopsy procedures for quantitative analysis of proteoglycans (GAGs) and sialoprotein (SA), though possible, are not a practical solution as patients invariably refuse biopsy. Therefore the circulating levels of sialic acid and GAGs in different orthopaedic disorders have been evaluated. Raised values of serum SA (*N*-acetylneuraminic acid) have been reported in osteoarticular tuberculosis,¹² chronic osteomyelitis,¹³ and rheumatoid arthritis.¹⁴

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Increased circulating levels of GAGs were demonstrated in patients with rheumatoid arthritis¹⁵ and in inflammatory conditions secondary to bacterial infection or traumatic injuries.¹⁶

These increases in SA and GAGs, however, had very little diagnostic significance when studied separately because the same pattern of change was noted in more than one condition. SA and GAGs have also been studied in bone metabolism relating to fluoride toxicity. For the first time the SA/GAG ratio was applied as a diagnostic test in osteofluorosis to assess the onset of toxic manifestations in bone as a result of excess ingestion of fluoride.¹⁷ A significant correlation was noted between the ratio SA/GAG and osteofluorosis when compared with normal values. The mean ratio was reduced to 30–50% in fluoride toxicity.¹⁷

It is in this context that the present study was undertaken to investigate whether the ratio of SA to GAG could be of diagnostic importance in other orthopaedic disorders.

Patients and methods

Cases were selected from orthopaedic outpatient clinics and from inpatients at the All India Institute of Medical Sciences, New Delhi. In each case a detailed clinical examination and careful radiological examination were done and the diagnosis confirmed. Five different types of bone disorders were chosen. Table 1 shows details of the cases investigated. The control group consisted of 10 healthy individuals selected from students, laboratory workers, and resident doctors (Table 1). Blood (5.0 ml) was obtained from each individual and serum samples were stored at -20°C . Sialic acid and GAG estimations were done within 48 hours.

ESTIMATION OF SIALIC ACID

Serum sialic acid was estimated according to Winzler¹⁸ with *N*-acetylneuraminic acid (Sigma, USA) as standard. A 4.80 ml aliquot of 5.0% trichloroacetic acid was added to 0.2 ml of serum in

capped test tubes and placed in a boiling water bath for 15 minutes. After cooling in ice cold water the samples were centrifuged at 1500 rpm for 15 minutes. Aliquots of the supernatant were used for colour development with diphenylamine reagent (1 g diphenylamine in 90 ml glacial acetic acid+10 ml concentrated H_2SO_4). Correction for non-specific colour development was applied. Optical density was read on a Spectronic-2000 spectrophotometer at 530 nm. The results were expressed as mg/l.

ESTIMATION OF GLYCOSAMINOGLYCANS

GAGs in sera were estimated according to Whiteman.¹⁹ To a known amount of serum (20 μl) in small disposable plastic tubes, 1.0 ml alcian blue reagent (0.05% w/v alcian blue 8GX and 50 mM MgCl_2 in 50 mM CH_3COONa adjusted to pH 5.8 with acetic acid) was added. The GAG-alcian blue complex (precipitate) was allowed to settle for four to six hours and separated by centrifugation at 2000 rpm for 15 minutes. The precipitate was washed with 2.0 ml ethanol and dissociated with 1.0 ml manaxol IB reagent (40% w/v manaxol IB in 50 mM sodium acetate buffer, pH 5.8). The clear solution was read at 620 nm in a Spectronic-2000 spectrophotometer. Chondroitin sulphate (from shark cartilage, Koch light) was used as standard. The results were expressed as mg/l.

STATISTICAL METHOD

The significance of the difference between the mean values was estimated by Student's *t* test.

Results

CONTROL GROUP

Eight men and two women aged 23–55 years (mean (SD) 32.9 (11)) were studied. Their serum sialic acid concentrations ranged from 648.0 to 812.7 mg/l (mean (SD) 722.2 (67.1)) and their GAG concentrations from 165.0 to 225.0 mg/l (mean (SD) 194.6 (18.4)). The ratio SA/GAG ranged from 3.0 to 3.97 (mean (SD) 3.73 (0.42)) (Table 2, Fig. 1).

Table 1 *Details of cases investigated*

Condition	Number of cases			Age range (years)	Average age* (years)
	Male	Female	Total		
Control	8	2	10	23–55	32.9 (11)
Osteofluorosis	4	2	6	19–60	37.3 (15)
Ankylosing spondylitis	16	1	17	16–50	31.0 (8.2)
Idiopathic backache	8	2	10	18–40	31.5 (8.2)
Osteoarthritis	4	12	16	40–60	50.0 (6.0)
Osteoporosis	1	19	20	32–70	53.0 (11.9)

*Values are means (SD).

Table 2 Results for sialic acid (SA), glycosaminoglycans (GAGs), and SA/GAG in the six groups investigated. Values are means (SD)

Condition	Sialic acid (mg/l)	Glycosaminoglycans (mg/l)	SA/GAG	SA/GAG range
Control	722.2 (67.1)	194.6 (18.4)	3.73 (0.42)	3.0-3.97
Osteofluorosis	484.5 (77.4)*	206.8 (43.7)	2.36 (0.24)*	1.91-2.58
Ankylosing spondylitis	895.9 (183.9)**	197.9 (34.4)	4.58 (0.95)**	3.72-6.32
Idiopathic backache	613.4 (159.3)	181.5 (28.6)	3.45 (1.03)	2.35-4.75
Osteoarthritis	568.2 (73.8)*	165.2 (58.9)	3.9 (1.55)	1.61-7.47
Osteoporosis	597.0 (91.9)*	185.8 (61.8)	3.67 (1.61)	1.71-7.47

* $p < 0.001$; ** $p < 0.01$.

ANKYLOSING SPONDYLITIS

Seventeen patients (16 male, one female) with ages ranging from 16 to 50 years (mean (SD) 31 (8.2)) were studied. Table 2 shows that the sialic acid concentration in serum increased ($p < 0.01$), whereas the GAG content showed no significant difference from the control values. For the ratio SA/GAG a significant increase was noted ($p < 0.01$) (Table 2, Fig. 1).

OSTEOPOROSIS

Twenty patients (19 female, one male) with ages ranging from 32 to 70 years (mean (SD) 53 (11.9)) were studied. The serum sialic acid concentration showed a significant decrease ($p < 0.001$), but the GAG concentration and the ratio SA/GAG showed no statistically significant change from control values (Table 2, Fig. 1).

OSTEOARTHRITIS

Sixteen patients (four male, 12 female) were included in this group. Their ages ranged from 40 to 60 years (mean (SD) 50.0 (6.0)). A significant decrease in serum sialic acid concentration was observed ($p < 0.001$). The GAG concentration showed a slight decrease, but the difference was not statistically significant. The ratio of SA to GAG remained unaltered (Table 2, Fig. 1).

IDIOPATHIC BACKACHE

Ten patients were studied (eight male, two female) with ages ranging from 18 to 40 years (mean (SD) 31.5 (8.2)). The sialic acid and GAG concentrations in serum showed no statistically significant difference, and the ratio SA/GAG was also unchanged when compared with control values (Table 2, Fig. 1).

OSTEOFUOROSIS

Six patients (four male, two female) with ages ranging from 19 to 60 years (mean (SD) 37.3 (15)) were studied. The sialic acid content in serum decreased, whereas the GAG concentration was slightly increased. The decrease in sialic acid concentration was significant ($p < 0.001$), but the increase in the concentration of GAG was not statistically significant. The ratio of SA to GAG showed a highly significant decrease ($p < 0.001$) when compared with the control value (Table 2, Fig. 1).

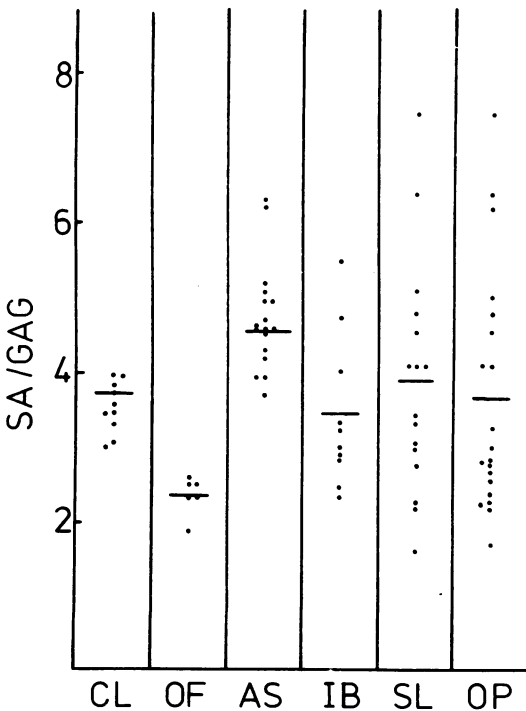


Fig. 1 SA/GAG ratio distribution in CL=control ($n=10$); OF=osteofluorosis ($n=6$); AS=ankylosing spondylitis ($n=17$); IB=idiopathic backache ($n=10$); SL=osteoarthritis ($n=16$); OP=osteoporosis ($n=20$). The horizontal bars show the mean for each group.

Discussion

Bone, being one of the most highly mineralised and

complex tissues in the body, is poorly understood in several aspects. The spectrum of non-collagenous proteins within the mineralised bone indicates that these molecules are necessary for matrix structure and mineralisation.²⁰

Sialoprotein (sialic acid) and proteoglycans (GAGs) are two very significant non-collagenous proteins of bone.²¹ A large portion of non-collagenous protein in developing bone is serum derived proteins,²²⁻²⁴ which indicates that these proteins are freely diffusing in the vicinity of the mineralisation front.²¹ Thus a link between serum proteins and non-collagenous proteins of bone is expected. It is in this context that the present study of sialic acid and GAGs was undertaken in serum.

An increase in serum sialic acid content was observed in AS, whereas in other bone diseases investigated (osteofluorosis, osteoarthritis, osteoporosis) a decrease was noted compared with control values (Table 2). Ninety per cent of sialic acid in normal sera is bound to α and β globulins, and these fractions of serum protein are known to increase in inflammatory conditions.¹² This, therefore, may be one of the reasons for enhanced serum sialic acid concentrations in AS.

The ESR is known to increase in inflammatory conditions, but in AS its relation with disease activity and duration of illness is in dispute. Blumberg and Regan found high ESR values in early stages of spondylitis but more normal values with longer disease duration.²⁵ Kendall *et al* reported no correlation between ESR and disease activity as assessed by pain scale.²⁶ Although in the present investigation raised values were found in 10 out of the 13 cases of AS investigated for ESR, the increase was not specific as 50% of the patients with osteoporosis and osteoarthritis also showed an increased ESR, thus establishing the fact that ESR is diagnostically unspecific for AS. (As a routine clinical test the ESR was investigated by the clinical pathology laboratory at the All India Institute of Medical Sciences in the same series of patients and it was observed that 10 cases of AS out of 13, eight cases of osteoporosis out of 16 and six cases of osteoarthritis out of 12 had raised ESR (ESR normal value ≤ 20 mm/1st h).

Patients with osteofluorosis and osteoporosis present divergent radiological manifestations, but both the groups, interestingly, show a similar decrease in their circulating levels of sialic acid (Table 2). The results suggest that possibly a common biochemical pathology is involved in these two conditions. A similar decrease in sialic acid is also noted in patients with osteoarthritis. The reduction in sialic acid concentrations observed may be due to either a decrease in the biosynthesis or the protein synthesis

may itself get disturbed, resulting in low circulating levels of protein bound carbohydrate.²⁷

Unlike sialic acid, serum GAG concentrations show no significant alteration from control values, except for a slight increase in osteofluorosis and a slight decrease in osteoarthritis (Table 2). The reason for this is not evident, though it may possibly be due to the fact that the percentage of GAGs present in bone is far less than the sialic acid fraction^{28, 29} or that the GAGs are degraded and instead of remaining in circulation are excreted.

Jha *et al* in a study on fluoride toxicity and fluorosis established a diagnostic test for osteofluorosis using the SA to GAG ratio.¹⁷ The same test was applied to five different orthopaedic disorders, including osteofluorosis.

It is an established fact that in idiopathic backache neither the clinical nor the radiological presentations are different from those of normal subjects. The results obtained from the present study also confirm the view that there is no significant change between idiopathic backache and normal subjects. Although patients with osteoporosis and osteoarthritis showed a decrease in their circulating levels of sialic acid, no difference was noted in their mean SA/GAG ratio (Fig. 1, Table 2).

A 37% decrease in the mean ratio of SA/GAG was noted in osteofluorosis when compared with control values. This is in agreement with a previous study.¹⁷

Ankylosing spondylitis and osteofluorosis have similar radiological presentations and clinically too the two conditions are somewhat similar,³⁰ but the mean ratio of SA/GAG was found to be nearly double that for osteofluorosis. The mean ratio in ankylosing spondylitis was also significantly different ($p < 0.01$) from the control value (Table 2, Fig. 1).

The SA/GAG ratio for the 17 patients with ankylosing spondylitis and the six with osteofluorosis matched with the data for 10 controls shown in the scattergram (Fig. 1) proves the precision and validity of the test. Ankylosing spondylitis may be undiagnosed or misdiagnosed because the clinical features vary considerably.³¹ An increase in ESR, CRP, and immunoglobulin concentrations and haematological changes like thrombocytosis and leucocytosis, though found to be associated with ankylosing spondylitis, are also noted in other inflammatory rheumatic diseases,^{3, 4} rendering the above test unspecific. Considering the above facts our findings are encouraging and the SA/GAG ratio is recommended as a diagnostic test for AS.

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