RESEARCH

Evaluation of mandibular bone mineral density using the dual-energy X-ray absorptiometry technique in edentulous subjects living in an endemic fluorosis region

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Objectives: Fluoride is one of the biological trace elements with a strong affinity for osseous, cartilaginous and dental tissue. The dental and skeletal effects of high fluoride intake have already been studied in the literature, but little is known about the effects of high fluoride intake on edentulous mandibles. The purpose of this study was to evaluate the effects of high fluoride intake on mandibular bone mineral density (BMD) measured by the dual-energy X-ray absorptiometry (DXA) technique in edentulous individuals with systemic fluorosis.

Methods: 32 people who were living in an endemic fluorosis area since birth and 31 people who were living in a non-endemic fluorosis area since birth (control group) participated in this study. Systemic fluorosis was diagnosed in the patients using the sialic acid (NANA)/glycosaminoglycan (GAG) ratio. The BMDs of the mandibles were determined by the DXA technique.

Results: The serum NANA/GAG ratios in the fluorosis group were significantly lower than those in the control group ($p < 0.001$). There was also a statistically significant difference in mandibular BMD measurements ($p < 0.05$) between the systemic fluorosis and control groups, as measured by the DXA technique. Mandibular body BMD measurements were higher in the fluorosis group ($1.25 \pm 0.24 \text{ g cm}^{-2}$) than in the control group ($1.01 \pm 0.31 \text{ g cm}^{-2}$).

Conclusions: The results of the study showed that fluoride intake higher than the optimum level causes increased mandibular BMD in edentulous individuals. Further dose-related studies are needed to determine the effects of high fluoride intake on bony structures of the stomatognathic system.

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Introduction

The bone mineral density (BMD) of the jaws is gaining importance in contemporary dental practice because mandibular BMD may have an important role in the treatment planning, management and prognosis of dental procedures such as osseointegrated implants, periodontal disease and grafting.^{1–6} Van Steenberghe et al⁷ emphasized how it is logical that bone volume and quality are factors of relevance in such procedures since

the principle of osseointegration is based on intimate bone-to-implant contact which is achieved during healing and maintained over the years, even under loading. It was also shown that surgical trauma and anatomical conditions are the most important factors for primary implant losses; jaw bone quality, volume and overload are major determinants for late implant failures.⁸ The impact of BMD of the jaws on implant prosthodontics and other dental procedures has led clinicians to investigate bone structure variations in different systemic conditions.

Systemic factors that effect bone metabolism seem to determine the final rate of resorption of edentulous alveolar ridges after the influence of local causes has

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decreased with time.^{9–11} Fluoride is one of the essential trace elements with a systemic action on bone that has been extensively studied. Among its characteristics, fluoride is mainly incorporated into calcified tissues, which means it can be found in teeth and bone because of its high affinity for calcium (these contain approximately 99% of the total body burden of fluoride).¹² It replaces the hydroxyl group of hydroxyapatite crystals to form fluoroapatite, which is less soluble and more compact. Fluoride increases metabolic turnover of the bone and stimulates bone cell proliferation. Because of these properties, the fluoridation of community drinking water supplies is employed for the prevention of dental caries and in the treatment of osteoporosis.¹³ Most of the relevant studies have reported 1 ppm (parts per million) of sodium fluoride to be a safe level in drinking water; however, a high intake of fluoride (>1.5 ppm) for a prolonged period is known to cause dental and skeletal side effects.¹³

Dental and skeletal fluorosis is an endemic public health problem in some regions of various countries around the globe.^{14–16} In southern Turkey, the city of Isparta is a severely endemic fluorosis region. The high fluoride intake means severe dental fluorosis is commonly encountered in Isparta.¹⁷ The natural water supply is the major source of fluoride ions in this city. Annual minimum and maximum fluoride levels of Gölçük Crater Lake and Andik River (the water sources of Isparta) are 0.79 ppm and 1.55 ppm (1.12 ± 0.29 ppm) and 2.55 ppm and 3.40 ppm (2.96 ± 0.31 ppm), respectively.¹⁸ The bony effects of high fluoride intake have been widely investigated in both clinical and laboratory studies;^{19–32} however, the authors of the present study did not find any reports in the literature relative to mandibular BMD in edentulous subjects with systemic fluorosis. The purpose of this study was to evaluate mandibular BMD in edentulous patients with systemic fluorosis diagnosed using the sialic acid (NANA)/glycosaminoglycan (GAG) ratio.

Materials and methods

The edentulous patients in the Prosthodontics Department of Suleyman Demirel University Faculty of Dentistry, Isparta, Turkey, were solicited by a written announcement to participate in this study. Edentulous patients who attended the clinics of the Prosthodontics Department to participate in the study and who had been living in an endemic fluorosis region since birth were subjected to anamnestic, radiological and clinical investigations. The effect of exposure to fluoridated water on the residence history of patients and their weight, height, calcium intake, lifestyle factors, numbers of years after menopause and medical history were assessed using a questionnaire. Patients who were being treated for conditions known to affect bone metabolism, such as hyperparathyroidism, renal disease and diabetes mellitus, were not included in the

study. None of the patients was smokers (ex-smokers a smoking period was >10 years) or drank excessive amounts of alcohol (≤ 2 servings per day). None of the patients had undergone dental surgical procedures except tooth extraction. Dental panoramic radiographs were used to determine the residual roots or any other pathology. All female patients had undergone a natural menopause. 32 patients (18 female and 14 male) were recruited to the study as the fluorosis group. After designation of the fluorosis group, the control group was determined from the existing patient files who had been living in a non-endemic fluorosis region since birth and consisted of 32 subjects with matching age, sex, period of edentulism and body mass index (BMI) with the fluorosis group patients. The fluorosis and control group patients were comparable with respect to age, gender and BMI (Table 1). The subjects were also similar socioeconomically. The study protocol was approved by the local ethics committee. Informed consent was obtained from all patients prior to their participation.

Morning arterial blood samples were collected from the fluorosis and control groups for NANA/GAG analysis. The NANA (Sialic Acid Quantitation Kit, Sialic-Q; Sigma-Aldrich, St. Louis, MO) and GAG (Wielisa GAG Quantitative Kit; Wieslab AB, Lund, Sweden) content in sera of the participants were estimated and fluorosis was diagnosed according to the method suggested by Jha et al.²²

Mandibular BMD (g cm^{-2}) was measured using dual-energy X-ray absorptiometry (DXA) (Norland XR-46; Norland Medical Systems Inc., Fort Atkinson, WI). The BMD measurements were performed on the body of the mandible as described by Horner et al.³³ The patients were positioned on their side with the neck extended to avoid superimposition of the cervical spine. Each mandible was scanned in a rectilinear manner starting from 1 cm above the temporomandibular joint through the whole of the mandible on one side. The image of the contralateral side was superimposed. In cases where both sides of the mandible were not superimposed because of a positioning error of the mandible, the scanning was repeated. Figure 1 represents the ideal and desired superimposition of the mandibular DXA image. After the DXA scan, images were recorded and displayed on the computer monitor; manual analysis of these scans was carried out using rectangular customized regions placed over the body of the mandible

Table 1 Patient characteristics

Characteristic	Patient group (n = 32)	Control group (n = 31)
Male:female	14:18	14:17
Age (years)	63.43 ± 8.05	59.93 ± 7.54
Period of edentulism (years)	17.72 ± 6.08	15.09 ± 3.76
Body mass index (kg m^{-2})	27.66 ± 2.50	28.16 ± 1.62

Data are given as ratio or mean $\pm$ standard deviation.

extending from the anterior ramus to the parasymphysis region. The size of the selected regions was adapted to conform to the shape of the mandible of each patient. The BMD (g cm^{-2}) of the selected region was calculated by lumbar spine computer software. Mandibular DXA scans were analysed by one independent investigator who was blind to the patient status to minimize interobserver variations. The reproducibility of the measurement system was assessed by repeating the analysis three times for each image. Intraobserver repeatability was tested with the concordance correlation coefficient. A one-sample *t*-test was used to compare the fluorosis group patients' NANA/GAG values with those of the control group. Independent samples *t*-test was used to compare the mandibular BMD values of fluorosis and control group subjects. An evaluation was made of the linear relationship between mandibular body BMD and NANA/GAG ratio using Pearson's correlation. The results were analysed using SPSS 13.0 (IBM Corporation, formerly SPSS Inc., Armonk, NY).

Results

There was no statistically significant difference between age, gender and BMI (Table 1) of the two groups. The years since menopause of the females showed no statistically significant difference between the fluorosis patients (17.83 ± 6.43 years) and control group (19.12 ± 6.49 years). One patient in the control group was unable to complete the study because it was not possible to get an ideal superimposition of the mandible. The mean serum NANA level was significantly lower in patients with fluorosis ($48.72 \pm 9.23 \text{ mg } 100 \text{ ml}^{-1}$) than in control patients ($68.04 \pm 8.46 \text{ mg } 100 \text{ ml}^{-1}$). An increased plasma

Table 2 Serum sialic acid (NANA mg/100 ml), glycosaminoglycan (GAG mg/100 ml) measurements and NANA/GAG ratios of the control and fluorosis group subjects

	Patient group (n = 32)	Control group (n = 31)
NANA (mg/100 ml)	48.72 ± 9.23^a	68.04 ± 8.46^a
GAG (mg/100 ml)	233.71 ± 54.44^a	193 ± 33.12^a
NANA/GAG(mg/100 ml)	0.194 ± 0.03^a	0.37 ± 0.037^a

^a*p* < 0.001.

Data are presented as mean $\pm$ standard deviation.

GAG level was observed in fluorosis patients ($233.71 \pm 54.44 \text{ mg } 100 \text{ ml}^{-1}$) compared with the control patients ($193 \pm 33.12 \text{ mg } 100 \text{ ml}^{-1}$). The mean NANA/GAG ratio was 0.194 ± 0.03 for the fluorosis group compared with 0.37 ± 0.037 for the control group (Table 2). There was a significant difference between the control group and the fluorosis group's NANA/GAG ratios (*p* < 0.001).

The mean BMD of the mandibles in the two groups can be seen in Figure 2. The concordance correlation for the intraobserver agreement were >0.90 for the mandibular BMD assessments. Thus, the mean of the three measurement results for each subject was used for the determination of the mandibular body BMD value. The mean mandibular body BMD measurement results are given in Table 3. The difference in the mandibular BMD values was statistically significant (*p* < 0.05). The mandibular BMD measurements were higher in the fluorosis group than in the control group. There was a very low correlation between NANA/GAG ratio and mandibular body BMDs both in the control group (*r* = 0.18) and fluorosis group (*r* = 0.14).

Figure 1 A representative dual-energy X-ray absorptiometry image of ideal mandibular superimposition

Figure 2 Box-plot diagram of fluorosis and control group's mandibular dual-energy X-ray absorptiometry measurements. BMD, bone mineral density

Table 3 Mandibular body bone mineral density (g cm^{-2}) measurements

Gender	Patient group (n = 32)	Control group (n = 31)
Male	1.28 ± 0.22	1.15 ± 0.32
Female	1.23 ± 0.27	0.90 ± 0.25
Total	1.25 ± 0.24^a	1.01 ± 0.31^a

^a $p < 0.05$.Data are presented as mean $\pm$ standard deviation.

Discussion

Fluoride has a strong affinity for hard tissues in humans. The fluoride concentration in bone is affected by high fluoride intake. While bone biopsies provide an accurate concentration of total body burden of fluoride,³⁴ they are unacceptable to patients. For the same reason, bone biopsies could not be performed to determine the effect of fluoride on mandibles in this study. Although the only real "gold standard" measurement of mandibular BMD would be bone biopsy, it is likely that DXA offers the best means of obtaining accurate information on BMD.³⁵ The DXA technique allows fast, non-invasive and highly precise measurements of BMD.³⁶ In this study, BMD measurements were performed on the body of the mandible since the body of the mandible gives BMD data that are most predictive of BMD in the hip, spine and forearm and which produces greater sensitivity and specificity compared with the ramus and the symphysis regions, as described by Horner et al.³³ The body of the mandible is also the location for implant placement, so the BMD of the ramus was not evaluated in this study. Superimposition is an important factor in the studies that use the DXA technique on mandibles. In cases where an ideal superimposition is not achieved, the obtained BMD values could either be half of the real mandibular BMD values or artificially high because of the superimposition of other bony structures. In the present study, the image was recorded onto a computer after the desired superimposition was observed on the screen. An ideal superimposition of the body of the mandible was obtained in all subjects.

GAGs and NANA are the important components of the bone matrix.³⁷ However, the changes in NANA and GAGs have unimportant diagnostic values when studied alone.³⁸ Thus, similar plasma NANA and GAG levels may be observed in more than one disease.³⁸ For instance, raised serum values of NANA have been noted in different cancers,³⁹ diabetic patients⁴⁰ and rheumatoid arthritis.⁴¹ Increased plasma levels of GAGs were reported in different cases such as bone tumours,⁴² heavy physical activities⁴³ and septic shock.⁴⁴ However, the NANA/GAG ratio was used for the diagnosis of fluorosis.²² It was found that after an excess fluoride intake, the mean NANA/GAG ratio was reduced to 30–50% when compared with patients that ingested optimum and low levels of fluoride. In this study, the NANA/GAG ratios of the fluorosis group ($0.194 \pm 0.03 \text{ mg } 100 \text{ ml}^{-1}$) were significantly lower than those in the control group ($0.37 \pm 0.037 \text{ mg } 100 \text{ ml}^{-1}$).

It is widely agreed that relatively high doses of fluoride increase bone density.^{25–27} In another study which investigated BMD after excessive fluoride intake in Isparta city, Yildiz et al¹⁹ showed that excessive fluoride intake via drinking water causes increased BMD values compared with normal patients. The mandibular BMD was also found to be higher in the fluorosis group which was similar to the results from a study conducted on femurs.¹⁹ These results were probably similar because the changes in bone metabolism that occur in the jaw region seem to be the same as those observed in long bones.

The increase in bone mass after excessive fluoride intake is due to increased remodelling activity with an unbalanced coupling in favour of bone formation.²³ It is generally accepted that there is a relationship between alveolar bone loss in edentulous patients and metabolic bone disease.^{4,5,23–25,45} Fluoride becomes incorporated into bone mineral either by fixing to the surface of the hydroxyapatite crystal or by exchanging with the hydroxyl ion on the surface or inside the crystal.¹² Gupta et al²⁴ showed that high fluoride intake affects the results of skeletal scintigraphy, including the calvaria and mandible, which both show increases in active bone formation. They concluded that increased bone turnover is present even in patients with a normal radiological picture and normal serum alkaline phosphatase levels. Klemetti et al²¹ showed that consuming artificially fluoridated water reduces the degree of residual ridge resorption. In a study that investigated the fluoridation and discontinuation of a fluoride profile in the alveolar bone of rats, of the parts of the crest (the middle, the apical parts of the alveolar bone and the body of the mandible), the upper parts of the alveolar bone and the alveolar crest part presented the highest rates of resorption owing to the discontinuation of fluoride.³¹ In the same study, the relative reduction rate of the fluoride concentration was closely related to the duration of discontinuation. Several studies showed that the BMD of the skeleton increased following the long-term administration of a greater than optimum level of fluoride.^{19,20,27}

The mandibular BMD was found to be $1.25 \pm 0.24 \text{ g cm}^{-2}$ in the study group and $1.01 \pm 0.31 \text{ g cm}^{-2}$ in the control group. In our previous study, the mandibular density was determined for chronic obstructive pulmonary disease (COPD) patients as $0.92 \pm 0.26 \text{ g cm}^{-2}$, with $1.16 \pm 0.28 \text{ g cm}^{-2}$ for the control group of the study.⁴⁵ The control group values in the present study are similar to the control group values of this former study but they are higher than the BMD results of the COPD patients. This can be explained by the fact that COPD may have caused osteoporosis in this group of patients. Devlin et al⁴⁶ showed mandibular BMD values of $1.11 \pm 0.3 \text{ g cm}^{-2}$ in their study. Our control group values were similar to those of this study; however, the fluorosis group in this study had higher BMD values. This result may have originated from the effects of fluoride on bone mineral content. Although the male and female samples were too small to get a

statistically significant result and the sample size might not justify the results, in the present study the mandibular body BMD difference between men and women was smaller in the fluorosis group than in the control group. The fluoridation of community drinking water supplies is also employed for the prevention of dental caries and in the treatment of osteoporosis.¹³ It was demonstrated that mandibular BMD assessed by DXA technique correlates significantly with BMD measurements of other important skeletal sites.³³ In the present study, fluoride may diminish the effects of osteoporosis/osteopenia in female patients in the fluorosis group so that the difference between men and women was smaller in the fluorosis group than in the control group patients.

In conclusion, a fluoride intake higher than the optimum level may cause increased mandibular BMD. Bone mineral density of the jaws seems to be of great importance, not only in primary implant stability, but

also in the predictability of oral implant outcomes. Most of the studies in the dental literature define bone quality as equivalent to bone density; however, the term "bone quality" includes bone metabolism, cell turnover, mineralization, maturation, the intercellular matrix and vascularity, amongst others. Thus, dose-related studies must be performed to investigate the effects of taking greater than optimum levels of fluoride on alveolar bone quality, quantity and structure of the jaws.

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References

1. Jemt T, Lekholm U. Implant treatment in edentulous maxillae: a 5-year follow-up report on patients with different degrees of jaw resorption. *Int J Oral Maxillofac Implants* 1995; **10**: 303–311.
2. Zarb GA, Schmitt A. The longitudinal clinical effectiveness of osseointegrated dental implants: the Toronto study. Part I: surgical results. *J Prosthet Dent* 1990; **63**: 451–457.
3. Jaffin RA, Berman CL. The excessive loss of Branemark fixtures in type IV bone: a 5-year analysis. *J Periodontol* 1991; **62**: 2–4.
4. Klemetti E, Collin HL, Forss H, Markkanen H, Lassila V. Mineral status of skeleton and advanced periodontal disease. *J Clin Periodontol* 1994; **21**: 184–188.
5. Tezal M, Wactawski-Wende J, Grossi SG, Ho AW, Dunford R, Genco RJ. The relationship between bone mineral density and periodontitis in postmenopausal women. *J Periodontol* 2000; **71**: 1492–1498.
6. Blomqvist JE, Alberius P, Isaksson S, Linde A, Hansson BG. Factors in implant integration failure after bone grafting: an osteometric and endocrinologic matched analysis. *Int J Oral Maxillofac Surg* 1996; **25**: 63–68.
7. van Steenberghe D, Quirynen M, Molly L, Jacobs R. Impact of systemic diseases and medication on osseointegration. *Periodontol* 2000 2003; **33**: 163–171.
8. Esposito M, Hirsch JM, Lekholm U, Thomsen P. Biological factors contributing to failures of osseointegrated oral implants. (I). Success criteria and epidemiology. *Eur J Oral Sci* 1998; **106**: 527–551.
9. Takaishi Y, Okamoto Y, Ikeo T, Morii H, Takeda M, Hide K, et al. Correlations between periodontitis and loss of mandibular bone in relation to systemic bone changes in postmenopausal Japanese women. *Osteoporos Int* 2005; **16**: 1875–1882.
10. Klemetti E, Vainio P. Effect of bone mineral density in skeleton and mandible on extraction of teeth and clinical alveolar height. *J Prosthet Dent* 1993; **70**: 21–25.
11. von Wövern N, Kollerup G. Symptomatic osteoporosis: a risk factor for residual ridge reduction of the jaws. *J Prosthet Dent* 1992; **67**: 656–660.
12. National Research Council. *Health effects of ingested fluoride*. Washington, DC: National Academy Press; 1993.
13. Jha SK, Mishra VK, Sharma DK, Damodaran T. Fluoride in the environment and its metabolism in humans. *Rev Environ Contam Toxicol* 2011; **211**: 121–142.
14. Pendrys DG. Fluoride ingestion and oral health. *Nutrition* 2001; **17**: 979–980.
15. Ishii T, Suckling G. The severity of dental fluorosis in children exposed to water with a high fluoride content for various periods of time. *J Dent Res* 1991; **70**: 952–956.
16. Savas S, Cetin M, Akdoğan M, Heybeli N. Endemic fluorosis in Turkish patients: relationship with knee osteoarthritis. *Rheumatol Int* 2001; **21**: 30–35.
17. Ertürk MS, Kirzioğlu Z. In vitro evaluation of dentin permeability of fluorotic primary teeth with a new electronic hydraulic conductance measurement system with photosensors. *Arch Oral Biol* 2007; **52**: 1057–1063.
18. Kir E. The determination and evaluation of nitrate, phosphate and fluoride levels in the sources of drinking water in Isparta city (postgraduate thesis). Süleyman Demirel University, Institute of Science, Department of Chemistry; 1996. (In Turkish).
19. Yildiz M, Akdoğan M, Tamer N, Oral B. Bone mineral density of the spine and femur in early postmenopausal Turkish women with endemic skeletal fluorosis. *Calcif Tissue Int* 2003; **72**: 689–693.
20. Yildiz M, Oral B. Effects of menopause on bone mineral density in women with endemic fluorosis. *Clin Nucl Med* 2003; **28**: 308–311.
21. Klemetti E, Kröger H, Lassila L. Fluoridated drinking water, oestrogen therapy and residual ridge resorption. *J Oral Rehabil* 1997; **24**: 47–51.
22. Jha M, Susheela AK, Krishna N, Rajyalakshmi K, Venkiah K. Excessive ingestion of fluoride and the significance of sialic acid: glycosaminoglycans in the serum of rabbit and human subjects. *J Toxicol Clin Toxicol* 1982; **19**: 1023–1030.
23. Boivin G, Meunier PJ. Fluoride and bone: toxicological and therapeutic aspects. In: Cohen RD, Lewis B, Alberti KGMM, Denman AM, eds. *The metabolic and molecular basis of acquired disease*. London, UK: Bailliere Tindall; 1990. pp. 1803–1823.
24. Gupta SK, Gambhir S, Mithal A, Das BK. Skeletal scintigraphic findings in endemic skeletal fluorosis. *Nucl Med Commun* 1993; **14**: 384–390.
25. Arnala I, Alhava EM, Kauranen P. Effects of fluoride on bone in Finland. Histomorphometry of cadaver bone from low and high fluoride areas. *Acta Orthop Scand* 1985; **56**: 161–166.
26. Boivin G, Chavassieux P, Chapuy MC, Baud CA, Meunier PJ. Skeletal fluorosis: histomorphometric analysis of bone changes and bone fluoride content in 29 patients. *Bone* 1989; **10**: 89–99.
27. Phipps KR, Orwoll ES, Bevan L. The association between water-borne fluoride and bone mineral density in older adults. *J Dent Res* 1998; **77**: 1739–1748.

28. DePaula CA, Abjornson C, Pan Y, Kotha SP, Koike K, Guzelsu N. Changing the structurally effective mineral content of bone with in vitro fluoride treatment. *J Biomech* 2002; **35**: 355–361.
29. Czerwinski E, Nowak J, Dabrowska D, Skolarczyk A, Kita B, Ksiezzyk M. Bone and joint pathology in fluoride-exposed workers. *Arch Environ Health* 1988; **43**: 340–343.
30. Ohmi K, Nakagaki H, Tsuboi S, Okumura A, Sugiyama T, Thuy TT, et al. The effect of fluoridation and its discontinuation on fluoride profiles in the alveolar bone of rat. *Calcif Tissue Int* 2005; **77**: 226–232.
31. Vieira AP, Mousny M, Maia R, Hancock R, Everett ET, Grynblas MD. Assessment of teeth as biomarkers for skeletal fluoride exposure. *Osteoporos Int* 2005; **16**: 1576–1582.
32. Rodríguez JP, Rosselot G. Sodium fluoride induces changes on proteoglycans synthesized by avian osteoblasts in culture. *J Cell Biochem* 2001; **83**: 607–616.
33. Horner K, Devlin H, Alsop CW, Hodgkinson IM, Adams JE. Mandibular bone mineral density as a predictor of skeletal osteoporosis. *Br J Radiol* 1996; **69**: 1019–1025.
34. Selwitz RH. Strategies for improving methods of assessing fluoride accumulation in body fluids and tissues. *Adv Dent Res* 1994; **8**: 111–112.
35. Horner K, Devlin H. The relationships between two indices of mandibular bone quality and bone mineral density measured by dual energy X-ray absorptiometry. *Dentomaxillofac Radiol* 1998; **27**: 17–21.
36. Denissen H, Eijssink-Smeets R, van Lingen A, van Waas R. Assessing mineral density in small trephined jawbone biopsy specimens. *Clin Oral Implants Res* 1999; **10**: 320–325.
37. Linde A, Jontell M, Lundgren T, Nilson B, Swanberg U. Non-collagenous proteins of rat compact bone. *J Biol Chem* 1983; **258**: 1698–1705.
38. Susheela AK, Das TK, Khurana JS, Jayaswal A, Dave PK. Circulating levels of sialic acid and glycosaminoglycans: a diagnostic test for ankylosing spondylitis. *Ann Rheum Dis* 1988; **47**: 833–837.
39. Dong X, Xu X, Han F, Ping X, Yuang X, Lin B. Determination of sialic acids in the serum of cancer patients by capillary electrophoresis. *Electrophoresis* 2001; **22**: 2231–2235.
40. Nayak SB, Bhaktha G. Relationship between sialic acid and metabolic variables in Indian type 2 diabetic patients. *Lipids Health Dis* 2005; **10**: 4–15.
41. Alturfan AA, Uslu E, Alturfan EE, Hatemi G, Fresko I, Kokoglu E. Increased serum sialic acid levels in primary osteoarthritis and inactive rheumatoid arthritis. *Tohoku J Exp Med* 2007; **213**: 241–248.
42. Velasco CR, Collic-Jouault S, Redini F, Heymann D, Padrines M. Proteoglycans on bone tumor development. *Drug Discov Today* 2010; **15**: 553–560.
43. Contini M, Pacini S, Ibba-Manneschi L, Boddi V, Ruggiero M, Liguri G, et al. Modification of plasma glycosaminoglycans in long distance runners. *Br J Sports Med* 2004; **38**: 134–137.
44. Nelson A, Berkestet I, Schmidtchen A, Ljunggren L, Bodelsson M. Increased levels of glycosaminoglycans during septic shock: relation to mortality and the antibacterial actions of plasma. *Shock* 2008; **30**: 623–627.
45. Buyukkaplan US, Akkaya A, Yildiz M, Bircan A, Aksoy Dogan A, Ozturk O. Mineral status of COPD patients under long-term inhaled corticosteroid therapy. *J Prosthodont* 2008; **17**: 462–467.
46. Devlin H, Horner K, Ledgerton D. A comparison of maxillary and mandibular bone mineral densities. *J Prosthet Dent* 1998; **79**: 323–327.