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Abstract

Abstract: Skeletal fluorosis is a systemic chronic disease caused by long-term intake of excessive fluoride, which accumulates in bone tissue and causes changes to the bone and periosteal tissue. Skeletal fluorosis is rarely considered in paleopathological analyses, but in areas with relatively high fluoride in the environment, it may be an important cause of bone hyperplasia. In this study, we observed pathological lesions consistent with fluorosis on two human skeletons from the Ming Dynasty (1368-1644 CE) excavated from a cemetery in western Henan Province. By using an electron microprobe to measure the fluoride content in the teeth, and by considering the living conditions in the area during the Ming and Qing dynasties, we conclude that the remains show evidence of skeletal fluorosis. We also consider the content of fluoride in the local groundwater, the local way of life, and other factors, to identify potential causes of skeletal fluorosis in this population and demonstrate that environmental factors were the main cause. This offers insight into the relationship between humans and their environments in China, especially western Henan Province, in the historical past. It also demonstrates the unique contributions bioarchaeology can make to environmental health studies and studies of the history of health.

Keywords: skeletal fluorosis; bone hyperplasia; Ming dynasty; environmental health; electron microprobe; bioarchaeology; archaeometry

1 Introduction

Though fluorine is one of the essential trace elements in the human body, excessive long-term intake leads to accumulation throughout the body and chronic toxicity (Xiao, 2008). This results in fluorosis, a type of systemic chronic aggressive disease that damages bone and the surrounding soft tissues, as well as teeth (Jiang, 2020; Lu et al., 2021). Fluorosis often occurs in specific geographical environments, namely, where people live for extended periods in high-fluoride environments.

Fluoride is absorbed by blood serum through the stomach and upper intestine, and then enters bone tissue. When the fluoride content in the body is too high, the hydroxyl group of hydroxyapatite in bone can be replaced by fluoride to form fluorapatite. Fluoride further replaces phosphate radicals to eventually form insoluble calcium fluoride, which is mainly deposited in bone, cartilage, articular surfaces, ligaments, and tendon attachment points, leading to bone sclerosis and increased bone density (Hou, 2013; Jiang, 2020; Liu et al., 2005). Excessive fluoride also leads to an imbalance in the processes of osteogenesis and osteolysis, resulting in osteosclerosis, osteoporosis, or both. In addition, excessive fluoride can consume a large amount of calcium, reduce the blood calcium level, stimulate an increase in parathyroid hormone, and cause bone decalcification or osteolysis. Clinically, this manifests as osteoporosis, osteomalacia, or even bone deformation (Yang, 2017). Because bone turnover is ongoing throughout life, and in the normal metabolism of bone, the tissue is broken down and its components are dissolved into the blood, reabsorbed by the body, or excreted through the kidneys (Largent and Heyroth, 1949; Franke et al., 1975), once the exposure to high fluoride is stopped, the damage to the body will be reduced or even disappear.

According to the research of domestic and foreign scholars on cases of skeletal fluorosis found in archaeological sites, the pathological characteristics of skeletal fluorosis are mainly as follows: Skeletal fluorosis affects the bones of the whole body. The specific manifestations are that the skull becomes thick and rugose, and the surface around the foramen magnum is roughened due to ossification of ligaments. Due to the ossification of the ligamentum flavum, ligamentum transversum, and other paravertebral ligaments, the spine may develop osteophytes, which in severe cases may even lead to vertebral ankylosis, and the ribs may develop osteophytes due to the ossification of the rib cartilage or ligaments. Ankylosis of the costovertebral joints and sternocostal joints may occur due to ossification of the costal cartilage or ligaments, which makes the ribs thick and rough. Ossification of the ligaments and tendon attachments of long bones occurs, especially at the femoral spine, radioulnar membrane, tibiofibular ligament, sacral tubercle, and sacrospinous ligament, with ankylosis in severe cases (Littleton, 1999; Hou, 2013, Huang 1999).

According to the different pathways of fluoride uptake by the human body, fluorosis can be divided into drinking water, coal burning, and tea drinking types (Zhao and Shen, 2000).

Drinking water fluorosis is widespread worldwide, with endemic areas distributed in more than 50 countries and regions including China, India, Russia, and the United States (World Health Organization, 2006). Coal burning fluorosis is unique to China, widely distributed in its alpine mountainous areas, with no reports abroad (Xie, 1990). The distribution of tea drinking fluorosis in China is relatively narrow, mainly in areas inhabited by ethnic minorities in the west where brick tea is commonly consumed. There are few reports of tea drinking fluorosis abroad (Sun and Liu, 2005). In terms of age distribution, the three types of fluorosis are basically the same, that is, children mainly experiencing dental fluorosis, adults experiencing both dental fluorosis and skeletal fluorosis, and older adults have the highest incidence of skeletal fluorosis (Zhao and Shen, 2000). Fluorosis caused by the different fluoride sources also has different manifestations in human bone. According to the results of modern endemic disease surveys, coal burning fluorosis is more severe than drinking water fluorosis. In general, the trabeculae of new bone are finer and denser in the case of coal burning fluorosis, and the incidence of joint damage is significantly higher than that of the drinking water type (Sun et al., 1994). Compared to drinking water and coal burning fluorosis, tea drinking fluorosis tends to be milder (Fan and Bai, 1998).

Epidemiologically, the incidence of fluorosis is higher in older individuals, since fluoride gradually accumulates in bones with age. Research shows that the typical age of onset of this disease is in young adulthood (Yang, 2017). In general, the disease tends to have greater expression in individuals with more physical activity (Nelson et al., 2016). However, genetic and dietary factors can influence the condition. For instance, magnesium, aluminum, and calcium can bind fluoride and prevent its absorption by the body (Nelson et al., 2016). Paleopathological research has found a higher incidence of skeletal fluorosis in men than in women (Hou, 2013; Littleton, 1999; Petrone et al., 2011), though research on modern areas with endemic fluorosis has found no significant sex differences (Fan and Bai, 1998).

Many scholars in the field of modern medicine have conducted research on endemic skeletal fluorosis, including on the pathophysiology, manifestations, and predisposing factors. However, cases of skeletal fluorosis reported in the paleopathological literature are rare (see Table 1). Cases of dental fluorosis were first found in skeletal samples from the Early

Neolithic and Chalcolithic Ages of Pakistan (Lukacs, 1984; Lukacs et al., 1985). Littleton (1999) identified paleopathological lesions consistent with skeletal fluorosis from the Delos period on the main island of Bahrain. A series of cases of skeletal and dental fluorosis were found in tomb excavations at Palmyra, Syria, initially observed macroscopically (Nakahashi, 1994; Nakahashi et al., 2001). This was later supported by chemical analysis, which found that the excavated teeth with lesions (n=7) had more fluoride than teeth without lesions (n=9), confirmed high fluoride concentration in local water sources, and curiously also found no correlation between fluoride content and age (Yoshimura et al, 2006). In 1997-1999, a very high percentage of skeletal remains found at Herculaneum showed evidence of skeletal and dental fluorosis (enthesophytes, fractures, osteoarthritis, and dental enamel mottling), indicating the condition was endemic in Roman times (Petrone et al., 2013). This was supported by histopathological changes, high bone fluoride concentration that increased with age (Petrone et al., 2011), and high dental fluoride in victims found at Herculaneum and surrounding towns (Petrone et al. 2019). Nelson et al. (2016, 2019) found skeletal lesions consistent with fluorosis, including osteosclerosis, enthesophyte formation, dental enamel defects, and a high incidence of fractures, on eight skeletons from the Woodland period Ray Site, Illinois (USA), which is located in an area that currently has high levels of environmental fluoride.

At present, there are few such findings reported within China. In the 1970s, small yellow pits were found on the crowns of the teeth of the human remains excavated at Tongzi, Guizhou Province (Wu, 1985), and at Xujiayao, Shanxi Province (Li and Qi, 1980). Signs of dental fluorosis were found in the 2,000-year-old human bone specimens found in Long County in 1991 (Jiang, 2007), and in 1992, seven cases of skeletal fluorosis were found in the Ganguya Cemetery in Jiuquan, Gansu (Zheng, 1992). In 1996, in the Dadianzi Cemetery, one individual was found to have suffered from skeletal fluorosis (Pan, 1996). In 2012, cases of fluorosis were reported in human bone specimens from the Warring States period and Qing Dynasty found in the Longkou Tombs of the Danjiang Reservoir area in Hubei (Zhou and Li, 2010); and in 2013, Hou described in detail skeletal fluorosis cases in human remains from the Yuci Ming-Qing period cemetery in Shanxi.

Table 1: The discovery and study of fluorosis

	Period	*Sites*	*Pathological manifestations*	*Source*
Other than China	Early Neolithic (7000-4800 BCE) and Chalcolithic (4800-2600 BCE)	Mehrgarh site in Baluchistan, Pakistan	Nine teeth had dental fluorosis	Lukacs, 1984; Lukacs et al., 1985
	2nd and 3rd century CE	Palmyra, Syria	25 individuals out of 33 (76%) in Tomb C and 45 individuals out of 65 (69%) in Tomb F had tawny plaques on the teeth; teeth with lesions contained more fluoride than those without lesions	Nakahashi, 1994; Nakahashi, 2001; Yoshimura et al., 2006
	79 CE	Herculaneum, Naples, Italy	73.5% of 76 individuals showed evidence of skeletal fluorosis; bones and teeth contain high concentrations of fluoride	Petrone et al., 2011, 2013, 2019
	Delos period (250 BCE-250 CE)	Main island of Bahrain	A total of 255 individuals, about 4% of the adult individuals had bone hyperplasia, about 44.8% had some degree of dental fluorosis	Littleton, 1999
	Middle and Late Woodland periods (55 cal BC-cal AD 495)	Western Illinois, USA	Eight of 117 individuals had skeletal and dental lesions consistent with fluorosis	Nelson et al., 2016, 2019
China	Middle Paleolithic (100-125 millennia BP)	Xujiayao site, Shanxi Province	The crown of one tooth had a prominent yellow pit	Li and Qi, 1980
	Middle Paleolithic (about 200 millennia BP)	Tongzi, Guizhou Province	The crowns of three teeth had distinct yellow pits	Wu, 1985
	~2000 BP	Long County, Shaanxi Province	Of the 832 permanent teeth, 87 had fluorosis	Jiang, 2007

1840-1600 BCE	Ganguya Cemetery in Jiuquan, Gansu Province	X-rays of vertebrae from 7 individuals showed characteristics of skeletal fluorosis	Zheng, 1992
4000-3400 BCE	Dadianzi Cemetery, Chifeng, Inner Mongolia	One individual showed characteristics of skeletal fluorosis	Pan, 1996
Warring States period (476-221 BCE) and Qing dynasty (1644-1912 CE)	Longkou Tombs of the Danjiang Reservoir area in Hubei Province	The teeth of six individuals showed tawny plaques, suspected to be dental fluorosis	Zhou and Li, 2010
Ming (1368-1644 CE) and Qing dynasties (1644-1912 CE)	Yuci Cemetery in Shanxi Province	Thirty individuals had signs of skeletal fluorosis and 49 individuals had signs of dental fluorosis	Hou, 2013

Multiple lines of evidence, including both macroscopic and microscopic bone changes and chemical analyses, are best used to confirm the presence or absence of fluorosis. In Iceland, for example, frequent volcanic eruptions are a source of fluoride in the environment, and specific eruptions in the past have been linked to deaths of livestock from fluoride poisoning (Walser et al., 2020). In one analysis, individuals who lived in the aftermath of an 18th-century volcanic eruption were analyzed for evidence for fluorosis, based on a contemporaneous account that suggested livestock and humans may have died from fluorosis after the eruption. However, a detailed analysis of three skeletons, including gross changes to bones and teeth, X-ray microanalysis and X-ray diffraction, histological analysis, and ICP-mass spectroscopy analysis of the presence of fluoride and other elements failed to find any evidence of fluorosis (Gestsdóttir et al., 2006). A later, more comprehensive analysis of 186 skeletons from seven sites across Iceland also looked for evidence of fluorosis in the country in historical periods (Walser et al., 2020). It found a high incidence of antemortem fractures, especially among men and those over 36 years of age, and some bone hypertrophy and enthesopathy, all of which is potentially consistent with skeletal fluorosis but also explainable by geography, activity, genetics, or diet. A wide range of fluoride content was found in the skeletons, and seemed to vary according to age, infectious disease status, dietary deficiencies, and heavy metal exposure, again reinforcing the importance of a holistic analysis in the diagnosis of fluorosis. In addition, diagenetic and other taphonomic processes must be considered when doing such multidisciplinary analysis. For instance, the complex effects of heat exposure on the bone histology of the victims of the eruption of Mount Vesuvius in AD 79, and their significance for the interpretation of the immediate impact of the eruption on human bodies, are still being debated (Martyn et al., 2020).

The aim of this study is to investigate two cases of possible fluorosis, in two individuals buried in the same tomb. The pathological manifestations on the bones were observed macroscopically, and the teeth were examined with the help of an electron microprobe to detect fluoride content. Finally, we investigated the fluoride content in the local groundwater to infer whether there was excess fluoride in the drinking water in the past. We also consider the living environment and way of life of the ancient residents of Zhangwan, to attempt to explain the causes of skeletal fluorosis in this area.

2 Materials and methods

2.1 Materials

The human bone material used in this article comes from the excavations of the 2020 Shanzhou District Zhangwan Township Shantytown Transformation Tomb Area Engineering Project. The terrain is high in the south and low in the north, and bordered on the east by a ditch, and on the west by the Menghua Railway. From October, 2020, to January, 2021, the Sanmenxia City Institute of Cultural Relics and Archeology conducted excavations. A total of 75 tombs were excavated, dating from the Spring and Autumn Period to the Ming and Qing Dynasties (8^{th} century BCE—19^{th} century CE). A total of 183 objects or fragments of pottery and bronze were unearthed (Henan Provincial Institute of Cultural Heritage and Archaeology, unpublished). Judging from the structure of the tombs and the funerary objects, the cemetery was not used by high status individuals.

Forty-three of the excavated tombs contained material dating to the Ming and Qing Dynasties. All were earthen chamber tombs with vertical shafts, and the burial goods included white porcelain bowls, black porcelain jars, and copper coins. Tomb M5 had a tomb shaft to the east of its tomb chamber, and one wooden coffin each on the east and west sides of the chamber, though they were poorly preserved. Each coffin contained one skeleton, also in a poor state of preservation. Funerary objects included black porcelain pots, white porcelain bowls, copper ornaments, and several copper coins, dating only to the Ming period (1368-1644). Both the individual on the east side (hereafter M5 East) and the individuals on the west side (hereafter M5 West) were only partly preserved (see Figures 1 and 2).

Tomb M5 is 2.6 meters long and 0.8-1.6 meters wide, and the tomb chamber has a small total area, with a simple shape and construction. In general, Ming tombs less than three meters long and less than 1.5 meters wide are considered small, and the tomb occupants likely to be low-ranking officials or commoner (He, 2019; Fu, 2022). Tomb M5 therefor probably belonged to commoners.

A molar from tomb M3 of the Dahuo site in Xiangyang, Hubei Province, was selected as a reference sample, because of the individual's comparable age and the low probability of fluorosis in this area. The individual from this tomb is a male, 30-35 years old at death, with only a small difference in age to the individuals in Zhangwan tomb M5. Twenty-two tombs from the Ming and Qing Dynasties were excavated at the Dahuo site, and 19 sets of human bones were unearthed. Some individuals suffered from oral diseases and osteoarthritis, but no osteophytes were found. In the 1980s, a survey of the main endemic diseases in rural areas of Hubei Province showed that the population of Dahuo Village, Dongjin Town, did not exhibit chronic fluorosis (Ma, 1987), so we speculate that the probability of endemic fluorosis in the historical population was also low.

2.2 Methods

First, biological sex (Phenice, 1969; Brothwell, 1981) and age at death (Lovejoy et al., 1985; Meindl et al., 1985; Meindl and Lovejoy, 1985; Wu et al., 1984) were estimated using macroscopic morphological changes. Pathological lesions on the bones were visually observed for the purpose of differential diagnosis, and X-ray imaging was used to observe fused joints (X-ray machine at Zhengzhou Central Hospital, suspension type single board X-ray direct digital imaging system, model MXHF-1500R, SN number 89077, equipment number G201810006941). According to the current diagnostic criteria for endemic skeletal fluorosis in China (WS/T 192-2021), typical pathological manifestations of skeletal fluorosis include an enlarged radial crest, with sclerotic edges and a rough surface; ossification of the obturator membrane; osteoporosis of the cortical bone at the attachment of the pronator teres; ossification of the soleus tendon; ossification of the sacrospinous ligament; and ossification of the sacrotuberous ligament (Chinese Center for Disease Control and Prevention, 2021).

Electron microprobe analysis is a common method in analytical chemistry, which can perform qualitative and quantitative analysis of a small area of a sample in situ without causing obvious physical and chemical damage, and which has high precision, high accuracy, and ease of analysis. In previous studies, electron microprobe analysis has only rarely been used to detect the fluoride content of bones, while it has often been used to test and analyze the fluoride content in teeth. Furthermore, fluoride will dissociate and diffuse in bone tissue, so the test results from teeth are likely more reliable (Littleton, 1999). Finally, we were not able to take enough bone samples to be representative of the skeletal fluoride content. Therefore, this paper uses teeth for fluoride detection, specifically, in the dentin. Dentin is located under the enamel of the tooth crown and constitutes the main body of the tooth. Dental enamel is fully formed within four to ten years after birth, and fluoride will not

The data were statistically analyzed using SPSS25 software. Data points from the three

Figure 1 Individual M5 East

individuals were basically normally distributed. Therefore, a single factor ANOVA was used to

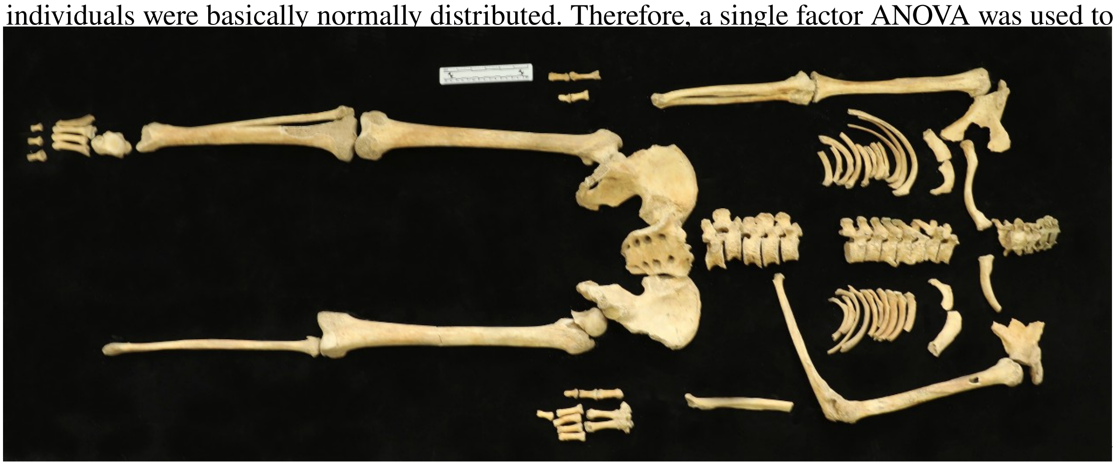

Figure 2 Individual M5 West

and ligaments has resulted in a rough surface of the ribs (Figure 3a) and sternum (Figure 3b).

The osteophytes in the lumbar spine and humeri are the most severe (Figures 3c-d). The four extant lumbar vertebrae are all involved, with thick osteophytes on the margins of the vertebral bodies, the laminae on both sides, and the ventral surfaces of the vertebral bodies, which is likely the result of ossification of the anterior longitudinal ligament and the insertion points of the erector spinae muscles. There is a thick osteophyte at the lesser tuberosity of the left humerus, which may be the result of ossification of the insertion point of the teres major and subscapularis muscles; the hyperplasia of the olecranon fossa of the right humerus and the slight osteophyte on the medial epicondyle are likely due to ossification of the origin of the pronator teres muscle (Figure 3d). There are slight osteophytes on the posterior superior iliac spine of the pelvis, which may be the result of ossification of the origin of the sacrotuberous ligament.

Individual M5 East has only 5 teeth, which are a maxillary premolar, and one mandibular canine and one mandibular premolar from each side. Clear linear enamel hypoplasia was seen on the tooth surfaces, the enamel transparency was high and the tooth surfaces were yellowish, and the enamel loss on the occlusal surface of the teeth exposed the dentin. The presentation of the teeth of this individual is therefore consistent with dental fluorosis, for instance, the loss of enamel on the occlusal surface of the teeth, though it cannot be ruled out that this is caused by abnormal wear of the teeth.

3.2 Individual M5 West

M5 West is estimated to be male and over 40 years of age at death. This individual has obvious osteophytes on almost all the bones, including severe pathological changes in the spine leading to ankylosis in the cervical and thoracic vertebral bodies. On X-rays of the cervical vertebrae, it can be seen that the ossification of the anterior longitudinal ligament and ligamentum flavum led to fusion of the vertebral bodies, the disappearance of the intervertebral disc space between the vertebral bodies, and an increase in trabecular bone. X-rays of the thoracic spine show ossification of the anterior longitudinal ligament and narrowing of the intervertebral disc space, also leading to fusion of the vertebral bodies (Figure 4), and relatively severe osteophytes in the long bones. Severe osteophytes appear on the anterior vertebral bodies, the margins of the vertebral bodies, and the articular surfaces. Two cervical vertebrae and two lumbar vertebrae have fusion of the vertebral bodies, possibly due to ossification of adjacent ligaments such as the anterior longitudinal ligament and ligamentum flavum. Severe osteophytes appear at the distal end of the left humerus and proximal end of the left ulna. X-rays show fusion of the humeroulnar joint and ossification of soft tissue on the posterior side of the elbow joint (Figure 5), resulting in ankylosis of the elbow joint, which coincides with the origin of the pronator teres and ossification of the brachialis muscle. Severe ossification and fusion have occurred in the right proximal and distal radius and ulna (Figure 6), which should be caused by ossification of the brachialis, pronator teres, and pronator quadratus muscles. There is ossification of the interosseous membrane of the ulna and radius, and a related slight periosteal reaction can be seen in the middle of the ulnar shaft on the X-rays. Severe osteophytes appear at both ends of the left metacarpals, and the proximal ends have fused with several carpal bones due to ossification. Blurred edges on cortical bone and trabecular bone can be seen on the X-rays (Figure 7). The linea aspera is thickened and the surface is rough, which may be the result of ossification of the insertion points of the adductor magnus, brevis, and longus muscles. Osteophytes appear at both ends of the left fibula (Figure 8), likely due to ossification of the origin of the soleus and the insertion point of the biceps femoris muscles. The surface of the fibular diaphysis is rough, possibly due to ossification of the tibiofibular interosseous membrane. The surface of the ribs is rugose, and osteophytes are evident on the lumbosacral articular surface on the sacrum, possibly due to ossification of the posterior sacroiliac and iliolumbar ligaments. Finally, there are slight osteophytes on the posterior superior iliac spine of the pelvis, which may be due to ossification of the origin of the sacrotuberous ligament.

Figure 6 M5 West right ulna and radius (A. ventral view; B. dorsal view; C. X-ray)

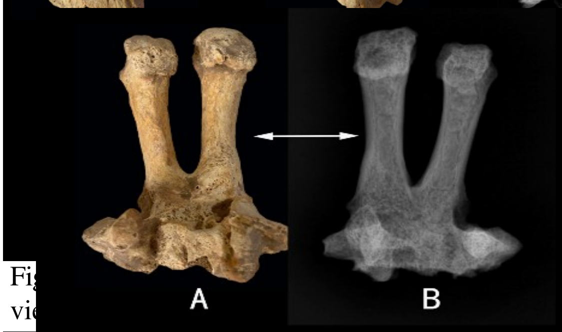

Figure 7 M5 West ankylosed metacarpal and carpal bones of the left hand (palmar view, A: photograph, B: X-ray)

Figure 8 M5 West left fibula: A. proximal epiphysis; B. midshaft; C. distal epiphysis (all posterior view)

ventral view), C)
view)

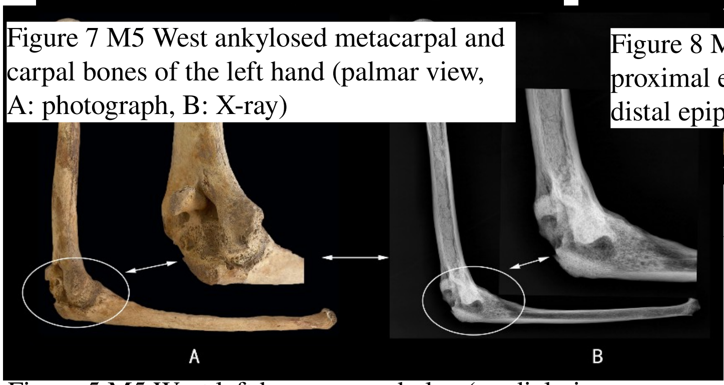

Figure 5 M5 West left humerus and ulna (medial view, A: photograph, B: X-ray)

3.3 Chemical analysis

The remains of the two individuals in this study were examined by means of electron microprobe. Teeth were selected based on preservation, and included a mandibular molar from Zhangwan M5 East and a premolar from Zhangwan M5 West. The comparative sample is a molar from tomb M3 of the Dahuo site in Xiangyang, Hubei Province, also from the Ming or

Qing period.

We excluded samples with values lower than 0.05 ppm, because samples below this level have low reliability. The results are presented in Table 2 and Figure 9. Table 3 shows the results of a one-way ANOVA comparing the data from the three individuals.

Table 2: Fluoride content in dentin in this study (wt %)

Individual	Dahuo M3	Zhangwan M5 West	Zhangwan M5 East
Fluoride content at sampling points (wt %)	0.081	0.088 _	0.087
	0.083	0.131 _	0.149
	0.109	0.137 _	0.196
	0.153	0.160 _	0.213
	0.155	0.198 _	0.257
	0.208	——	0.368
Average value	0.132	0.143	0.212

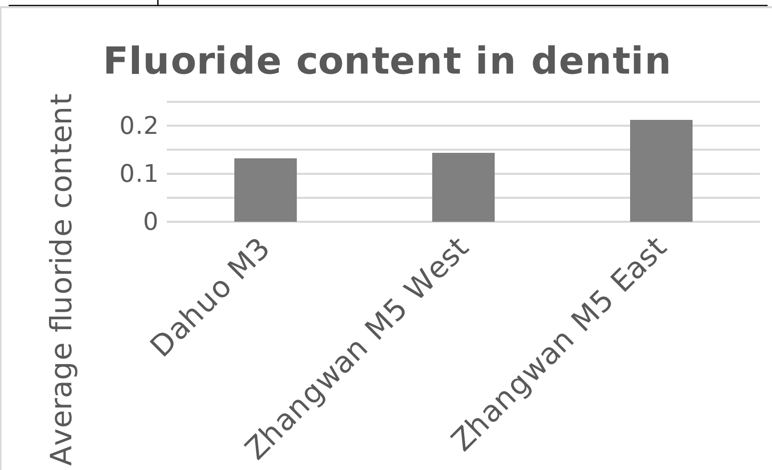

Figure 9: Fluoride content in dentin in this study (wt %)

Table 3: Comparison of fluoride content in this study ($\acute{X}$±s, wt%)

Individual	Fluoride content range (wt %)	F value	Significance
Dahuo M3	0.132 ± 0.045	2.391	0.128
Zhangwan M5 West	0.143 ± 0.036		
Zhangwan M5 East	0.212 ± 0.088		

As shown in Table 3, the two individuals from Zhangwan both have higher flouride content in their dentin than Dahuo individual M3, and the fluoride content of Zhangwan M5

East appears to be considerably higher. However, the one-way ANOVA found a P value greater than 0.05, so we cannot reject the null hypothesis that there is no difference between the three individuals.

4. Discussion

4.1 Differential diagnosis

There are six conditions that can cause pathological features similar to the hyperostosis seen on the bones from Zhangwan and that should be considered in a differential diagnosis, namely: osteoarthritis and vertebral osteophytosis, Paget's disease, developmental field anomalies, ankylosing spondylitis, diffuse idiopathic skeletal hyperostosis (DISH), and skeletal fluorosis.

Osteoarthritis is most common in individuals over the age of 50 and most often results in damage to the weight-bearing joints, namely the vertebrae, hips, and knees (Tao, 2008). It is characterized by sclerotic bone on joint surfaces or any two other features, such as osteophytes at the joint edge, new bone on the joint surfaces, pitting on the joint surfaces, and joint deformation. The condition does not generally lead to ankylosis (Waldron, 2008). Vertebral osteophytes also rarely cause ankylosis, and likewise tend to affect isolated joints rather than entire bones. There is also no generalized ossification of tendons and ligaments, as observed in the Zhangwan individuals (Bullough and Vigorta, 1984).

Paget's disease is characterized by increased bone remodeling, bone hypertrophy, and abnormal bone structure. The whole body can be affected, but the early lesions generally do not affect the whole body. The skull, pelvis, femora, spine, tibiae, and humeri are often involved. Ossification of tendons and ligaments is absent. It is regional and rarely seen in Asia (Li and Li, 2017).

Fusion, or rather, failure to separate during development, can occur in some limb segments and produce a similar appearance to some of the features of M5 West. For example, carpal coalition is the lack of separation between one or more carpal bones during development, as well as between carpal and metacarpal bones. In the case of ulnar hemimelia, the elbow can also be ankylosed at a flexed angle, in which case the humerus is reduced in size and fused with the proximal radius. Radio-ulnar synostosis results in the proximal radius and ulna not separating during development (Barnes, 2012). However, the lesions in M5 West are not entirely consistent with these conditions. For one, osteophytes at the distal ends of the fused metacarpals would not be explained by carpal coalition. It is the ulna, not the radius, that is fused with the left humerus, and the humerus is of a normal size and morphology. Finally, radiography shows the right radius and ulna do not share a medullary cavity as they would in radio-ulnar synostosis. There are also other hypertrophic lesions in both individuals that would not be explained by these isolated developmental anomalies.

In sum, osteoarthritis/vertebral osteophytes, Paget's disease, and developmental field anomalies all tend to affect isolated joints or bones, and generally do not have the characteristics of a systemic attack. They also do not include ossification of tendons and ligaments. Because both individuals in Zhangwan tomb M5 have ossification of cartilage, tendons, and ligaments, and have lesions involving almost the whole body, these diseases and developmental anomalies can be ruled out.

Ankylosing spondylitis mainly affects males in late adolescence or early adulthood (Riley et al., 1971). The onset is usually in the sacroiliac joints, extending up the spine, and in extreme cases it can also affect the ribs, with squaring of the anterior of the vertebrae, known as a "bamboo spine." Ankylosing spondylitis is characterized by thin, vertically orientated osteophytes forming in the spinal ligaments, and fusion of the apophyseal joints and sacroiliac joints. Fusion of the sacroiliac joints is the key point in the differential diagnosis of ankylosing spondylitis (Roberts and Manchester, 2010; Ye and Cui, 2004; Zhang, 1995; Zhu, 2011). In clinical samples, about 20% of cases had peripheral joint involvement, mainly in the lower extremities, including the metatarsophalangeal joints (Manchester, 1982). The vertebral bodies, ribs, and long bones of the M5 individuals had thicker osteophytes, and no squaring was found in the anterior vertebral bodies, nor did fusion of the sacroiliac joints appear, so ankylosing spondylitis can be ruled out as well.

DISH tends to affect the spine, and "candle wax" or "flowy" osteophytes appear, usually along the right anterolateral aspect of the thoracic spine. These changes can spread to the lumbar spine or even the cervical spine, and the pelvis, proximal femora, knee joints, and calcanei can all be affected (Utsinger, 1985). A diagnosis can be made by observing fusion of four consecutive vertebral bodies. Osteophytes will occur at the attachment sites of ligaments and tendons, which is also essential for a diagnosis (Roberts and Manchester, 2010). Skeletal manifestations are generally limited to the spine and lower extremities, ossification of costal cartilage is rare, and bone density generally does not change. DISH usually occurs in older male individuals (El Garf and Khater, 1984). Osteophytes in the vertebrae of the Zhangwan individuals appear on the margins of the vertebral bodies, the spinous processes, and both sides of the lamina of the vertebral arches, and were quite different from the "candle wax" or "flowing" appearance typical in DISH. In addition, DISH generally involves only the spine and lower extremities, while the lesions observed at Zhangwan occurred throughout the bodies, involving the spine, ribs, and upper and lower limbs, most notably in M5 West, whose left humerus and ulna and right ulna and radius were all involved.

According to the current diagnostic criteria for endemic skeletal fluorosis in China (WS/T 192-2021) (CEDC et al., 2021), typical pathological manifestations of skeletal fluorosis include enlargement, sclerotic margins, and increasing rugosity of the interosseous crest of the radius; ossification of the interosseous membrane of the lower leg; ossification of the obturator membrane of the pelvis; porosity of the cortical bone at the attachment of the pronator teres; ossification of the soleus tendon; ossification of the sacrospinous ligament; and ossification of the sacrotuberous ligament.

The individuals in Zhangwan tomb M5 showed ossification of tendon and ligament attachments involving the whole body. In this case, the ribs have a rough surface; the vertebrae, especially the lumbar and cervical vertebrae, have severe osteophytes leading to fusion of the vertebrae; the surfaces of the radius and ulna were rough; the radioulnar and tibiofibular interosseous membranes were ossified; the pronator teres and soleus tendons were ossified; and the sacrospinous ligament and sacrotuberous ligament were ossified. All together, this fits the paleopathological features of skeletal fluorosis and the current identification standards for skeletal fluorosis. After considering other conditions with similar lesions, this presentation is most similar to the pathological changes caused by skeletal fluorosis.

4.2 Detection of fluoride content

According to our analysis of dentin, the fluoride content in Zhangwan individual M5 East was the highest, followed by Zhangwan M5 West, and then Dahuo individual M3. This supports our hypothesis that the Zhangwan individuals were exposed to flouride during their lifetimes. However, the pathological lesions in M5 West are more serious than those in M5 East, though M5 East had the higher fluoride content. For instance, the spine and elbow joints of M5 West are ankylosed due to more severe ossification. This difference may be due to the physiological differences between individuals. The clinical manifestations of fluorosis are diverse and complex, and the intra-organismal environment of the body is also significantly different among patients, such as the sensitivity of the body to fluoride, the mechanical load on the bone at various sites, and the intake of vitamin C, vitamin D, calcium, and protein (Guizhou Medical College, 1986). The bones of M5 West are also poorly preserved, so the full extent of the bony pathology may not be evident.

The results of the ANOVA on the three individuals showed that there is no statistically significant difference in the sample means between the individuals (Table 3), so we cannot rule out the null hypothesis that there was no difference in the mean fluoride content of their dentin. However, the test was performed on a relatively small sample size. Further testing, with a larger sample size and better preservation, may find a significant difference.

4.3 Causal factors of skeletal fluorosis

Endemic fluorosis in China can be divided into three types, depending on the source of fluoride: drinking water fluorosis, coal burning fluorosis, and tea drinking fluorosis (Liu et al., 2016). Tea drinking fluorosis mainly occurs in areas inhabited by ethnic minorities who have the habit of drinking brick tea, primarily in western China (Sun and Liu, 2005), so this paper only considers the first two types.

Drinking water fluorosis is caused by drinking water with high fluoride content for a long time. Most of Shanzhou District is hilly and mountainous, with the Qinling Mountains in the south and the Yellow River in the north. The geological structure is complex, and the rock formations are completely exposed. This geology creates a risk of endemic drinking water fluorosis (Chen, 2003). Shanzhou District invests a lot of money every year in water improvement and defluoridation to prevent and control endemic fluorosis. However, tests of fluoride content in rural drinking water in Sanmenxia City from 2004 to 2005 showed that there were still a large number of villages in Shanzhou District with fluoride content in water exceeding a safe standard (Luo, 2011). Another government report included tests of water resources at three locations in Shanzhou District, one of which had a fluoride content of 1.14mg/L in the dry season and as high as 1.3mg/L in the wet season, also exceeding the safe standard (SCWCB, 2002). The fluoride content in the water was higher than the normal value in both the wet season and the dry season, indicating that it was minimally affected by rainfall and groundwater levels. Unless there was recent large-scale geological erosion, the fluoride content would not change significantly between historical periods and today. Therefore, during the Ming period, when the M5 individuals lived, the fluoride content of water in Shanzhou District was also likely elevated.

In addition to fluoride in water, other chemical components can also affect fluorosis prevalence. Experiments in animals have shown that calcium and magnesium can reduce the absorption of fluoride and promote the excretion of fluoride from the body (Reddy, 2009), while sodium and higher alkalinity will promote the absorption of fluoride (Pinet and Pinet, 1968). The dissolved salts in the groundwater near Zhangwan Township are mainly Na^+, Ca^{2+}, HCO_3^-, and SO_4^- . In recent survey results of groundwater in Sanmenxia City, pH values from the three points in Shanzhou District in the wet season and the dry season were all greater than 7, which is alkaline. In the groundwater 200 m to the east of the Yuandian Town railway station, fluoride content not only exceeded safety standards, but the pH value in the wet season also reached 8.3 (SCWCB, 2002). The combined conditions of high fluoride content, high sodium content, and high alkalinity in groundwater sources could lead to endemic fluorosis in the area.

Coal burning fluorosis is caused by residents burning high-fluoride coal, in an outdated method of stacking coal for heating and cooking (Cao, 1991a). When burning coal with high fluoride content, a large amount of gaseous fluorine and fluoride-containing soot will be emitted, and residents who inhale high concentrations of fluoride and other coal-burning pollutants may develop fluorosis (Cao, 1991b). In addition, even if residents use low-fluorine coal, the geographical environment, outdated coal-burning methods, poor living conditions, poor indoor ventilation, and other factors can also lead to coal burning fluorosis (Gao et al., 1992). Sanmenxia City is rich in coal resources. During the Ming and Qing dynasties, coal mining activity in western Henan was already considerable (Wang, 1996). According to records, coal was already produced in Shanzhou in the Ming Dynasty (Tangyin County Chronicles, 1637). There were ten coal kilns in Shanzhou by the tenth year of the Qianlong reign of the Qing dynasty (1745) (Appendix Memorial of the Military Aircraft Department, Qing Dynasty), and the use of coal had become a daily necessity. Finally, due to the unique geographical conditions in Shanzhou, the local residents have long had domestic architecture dominated by courtyard cave homes, with both cliff-style and free-standing cave dwellings. The cave dwellings are relatively enclosed, with windows on only one side and poor ventilation (Qin, 2010). Therefore, the use of coal in this area may also have been one of the causes of fluorosis.

In addition, studies in India have shown that manual laborers are more likely to develop skeletal fluorosis (Pandit et al., 1940). The reason may be that manual laborers tend to need to consume more water and their joints are prone to strain and trauma due to years of work, and the formation of new fluorinated bone seems to occur in those parts of the body most vulnerable to strain and minor trauma (Jolly et al., 1969; Wang et al., 1994). The society of the Ming period was still dominated by a small-scale peasant economy combining farming and weaving (Wang, 2000), and the male occupant of Tomb M5 was not a high-status individual, who may have needed to undertake heavy agricultural labor or manual labor, and therefore might have been more likely to suffer from skeletal fluorosis.

Finally, local climate might have contributed to the occurrence of fluorosis. The western part of Henan belongs to the north temperate continental monsoon region, the climate is relatively dry, and the average annual rainfall is only about 500 mm. During the Ming and Qing dynasties, residents in western Henan used to dig pools and dry wells to collect rainfall during the water-scarce season to solve their domestic water difficulties. Residents in the north also had the habit of using water tanks to store water for domestic use (Hu, 2007). The higher the temperature, the higher the evaporation rate of water, and the higher the concentration of fluoride in the accumulated domestic water. People also tend to consume more water in hot and dry climates, leading to even more consumption of fluoride.

4.4 Limitations of the study

Due to the impact of the burial environment and severe looting, the human bones from the Zhangwan cemetery are poorly preserved. Only the two skeletons from tomb M5 are relatively complete and have clear bone hyperplasia. This has led to a small sample, which cannot be confirmed as representative. Poor preservation also increased the difficulty of distinguishing the pathological manifestations on these two skeletons. This makes it difficult to discuss the wider prevalence and impact of skeletal fluorosis in the local population during the Ming and Qing dynasties.

As for this study's use of the electron microprobe for detecting fluoride content in the teeth, few previous studies have used this method to detect fluoride content in ancient human bones, so there is no appropriate standard value of normal fluoride content for comparison among other ancient populations. Therefore, a comparative sample had to be selected for the purposes of this study. We selected a skeleton without pathological manifestations of fluorosis, from an area without modern or archaeological evidence for fluorosis, to use as a reference sample to compare with the fluoride content of the Zhangwan M5 individuals. We also hope that our test results can be used as a reference in future studies.

In addition to drinking water, an individual's daily diet also affects the intake of fluoride. No stable isotope analysis or other direct dietary reconstruction was performed on the Zhangwan M5 human bone material. Therefore, it is not possible at this time to discuss the relationship between the fluoride content in the M5 individuals' bones and the local diet.

5. Conclusion

In this paper, pathological lesions on two sets of human skeletal remains from the Ming dynasty in the Zhangwan area of Shanzhou were examined and a differential diagnosis performed. It was concluded that they were most consistent with skeletal fluorosis. An electron microprobe was used to measure the fluoride content in tooth samples from these individuals, and the results showed that the fluoride content in the teeth was higher than that of a comparative sample. Though the difference was not statistically significant, the results provide context for judging the etiology of the pathological condition. Finally, factors in the local environment that might induce skeletal fluorosis were discussed, including groundwater contamination and the use of coal for heating and cooking.

Zhangwan is located in the Central Plains, where settled agriculture is the main subsistence mode, which also may have exacerbated both the occurrence and the impact of skeletal fluorosis. The clinical manifestations of skeletal fluorosis include long-term, persistent joint pain, joint movement limitations and motor dysfunction, reduced ability to work, and difficulty in caring for oneself (Huang, 2014). Patients with mild symptoms will have at the very least persistent discomfort, and those with severe symptoms will have decreased ability to work or even paralysis. The disease also tends to occur in young and middle-aged people, who are often the main labor force in the smallholder economic system. Daily life for those affected may require reliance on social support or government programs. Endemic fluorosis would therefore undoubtedly have caused a heavy economic burden to a family unit or even the whole area in historical periods.

The people buried at the Zhangwan cemetery in Shaanxi Province almost certainly drank local water with a high fluoride content, and probably experienced severe coal pollution and heavy physical labor, so the probability of endemic fluorosis was high. It is clear that endemic fluorosis seriously affects the health, working ability, and quality of life of people in high-fluoride areas. Therefore, the study of the paleopathology of skeletal fluorosis and the judgment of whether an ancient population suffered from skeletal fluorosis is of significance for reconstructing daily life and the social environment, and for understanding the social and economic systems of the time.

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Conflict of interest statement

The authors declare no conflicts of interest.

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