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RESEARCH ARTICLE

NANOINDENTATION MEASUREMENT ON INTERSTITIAL AND OSTEON OF BONE WITH OSTEOGENESIS IMPERFECTA-TYPE III

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ABSTRACT

Osteogenesis imperfecta (OI) is one of the genetic disorder which was characterized by bone fragility. Previous studies reported that there are several mechanical properties has been used to investigate the strength of OI bone. However, little data is yet available to describe bone material properties in individuals with this disorder for type III alone. Therefore, the aim of this study is to investigate the mechanical properties in individual with OI bone type III at interstitial and osteon. Bone tissue reduced modulus and hardness were measured by nanoindentation in three specimens with total 40 indents. These properties were compared between osteogenesis imperfecta types III for interstitial and osteonal microstructural regions. Individual with osteogenesis imperfecta type III had higher hardness and reduced modulus at interstitial rather than osteonal bone regions. Overall, the mean and standard deviation of hardness is 0.6 ± 0.11 GPa, while 0.44± 0.06 GPa for osteon. The current study presents the dataset describing the bone material properties in individual with Osteogenesis Imperfecta Type III. Results indicate that intrinsic bone tissues properties were affected by phenotype. Knowledge on the mechanical properties of severity osteogenesis imperfecta may help to assist the model and prevent the fracture risk for those having this disorder.

KEYWORDS

Osteogenesis imperfecta, mechanical properties, reduced modulus, hardness, nanoindentation.

1. INTRODUCTION

Osteogenesis imperfecta (OI) is a heterogeneous collagen-related genetic disorder resulting in bone fragility [1]. Bone fragility in OI consisted of the combination of bone mass deficiency and bone tissue material properties [2]. There are four subdivisions of OI bone which are type I, II, III and IV. The most and common occur in the early birth is type I, that is associated with loose joints (hyperextensible), low muscles tone (hypotonia) and thin skin that bruises easily. OI type II is a severe complication occur in infant which describes physically have low birth weight, abnormally short arms and legs (limbs), and bluish discoloration of the whites of the eyes (blue sclera). OI Type III characterized by fragile bones, multiple fractures, malformed bones and usually may have a triangular facial appearance due to an abnormally prominent forehead (frontal bossing) and an abnormally small jaw (micrognathia). OI type IV described the bones are fragile and often fracture easily, more common before puberty [3].

Commonly, it can be noted that the abnormalities of OI bone is related to the structural and material. From a structural perspective, person with OI bone tend to have low bone mass compared to normal bone. Several clinical analyses have reported that individual with OI also have lower cortical thickness and decreased trabecular bone per tissue volume in children with OI [4], [5]. Regarding to the mechanical properties, [6], [7] has been reported that OI type I had higher modulus and hardness than those in type III. Other researchers also justified that the degraded in mechanical properties was related due to the bone mineral and the organic matrix of bone content. In addition, the OI bone also has abnormalities in collagen and bone mineral crystals which is intrinsic such as tissue-level, and bone material behaviour. There are several past studies to investigate severity of OI bone by using nanoindentation test [3], [7]. Nanoindentation test is a technique used to probe a sample's intrinsic mechanical properties with 1μm localization resolution and nanolevel load resolution [8]–[10]. Since nanoindentation test could obtain intrinsic mechanical properties at the lamellar level without structural influences, therefore these technique measurements are usually used for intrinsic mechanical properties of material [11].

Modulus and hardness also common measurement enabling the strength of OI bone. These measurements were found to be higher in children with severe OI bone than age-matched controls [10]. However, no significant differences were reported between moderate and severe OI bone. No data is yet available to describe bone material properties on individuals with the most common form of OI type III only. It is worth noting that the range of modulus and hardness were (11-24 GPa) and (0.3-0.9 GPa) respectively [7], [12]. In previous studies also reported that these properties tend to be higher in interstitial than osteon bone regions [12], [13]. The aim of this study was to investigate the mechanical properties for OI bone type III by using nanoindentation technique.

2. MATERIALS AND METHODS

2.1 Specimens

Three bone specimens were collected from osteotomy of the femur of 19 years old OI male. These bone specimens were obtained from Orthopaedic Department of Hospital Universiti Sains Malaysia, Kubang Kerian, Kelantan. The bones sections were washed with deionized (DI) water at room temperature in approximately 10 min to avoid deterioration of bone and stored in a freezer at -70°C. The osteotomy of the femur was cut into three specimens that contained lamellar microstructure without appearance of callous tissue as shown in Figure 1.

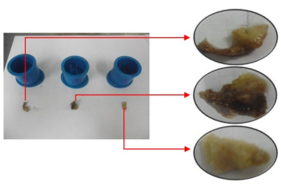

Figure 1: Three bone specimens obtained from the osteotomy of the femur with OI

2.2 Specimens preparation

All specimens were cut into cross-sectional area by using a diamond saw (Polycrstalline Diamond Slurry water base, 3nm, PC-1-w and PC-3-w) under constant water irrigation according to the required size and prepared for embedded. The cross-sections of bone specimens were oriented into the exposed surface approximately in perpendicular to the longitudinal axis of the long bone. After the cutting process, the specimens were fixed and dehydrated in graded ethanol solutions 70%, 24 h; 80%, 2 h;95%, 2 h; 95%, 3 h; 100%, 2 h; 100%, 2 h; and 100%, 3h.

In order to complete dehydration process, the specimens were air-dried in 5 min and embedded under vacuum in room temperature (Epokwick^{Tm} Epoxy Hardener, Buehler, Lake Bluff, IL, USA) and polymerization. After polymerization, performed the treatment for specimen surfaces by using grinder-polisher in order to get smooth surface before conducting nanoindentation test. The specimens were grounded using progressively fine grit sizes 400, 600, 800 and 1200. The specimens surface was polished by using polisher (Metaserv® 3000; Buehler, Lake Bluff, IL, USA). The surfaces were polished using a 3 µm aluminum oxide coated disc (Fibrmet®; Buehler, Lake Bluff, IL, USA). Final polishing was done with a polishing cloth (Microcloth®; Buehler, Lake Bluff, IL, USA) and a 0.05 µm alumina suspension (Micropolish® B; Buehler, Lake Bluff, IL, USA). After completed the surface treatment, the testing of nanoindentation will be performed on the specimens.

2.3 Experimental Procedure

Nanoindentation testing was performed using a nanoindenter (Nano Indenter XP; MTS, Eden Prairie, MN, USA) with Berkovich diamond indenter tip to measure elastic modulus, E and hardness, H as shown in Figure 2. These measurements were obtained via Continuous Stiffness Measurement (CSM) algorithm that was linked with the nanoindenter with a low magnitude oscillating force superimposed onto the nominally increasing load. Several variables such frequency, amplitude, strain rate, and maximum depth were defined during penetration between indenter and the specimen surface. Thereafter, continuously measure the modulus and hardness as a function of penetration depth by using the CSM algorithm. Frequency and amplitude were set at 45 Hz and 2 nm, respectively, as was done in previous studies [9], [14]. The hardness-depth were obtained at the constant strain rate of 0.05 s^{-1}, and a depth limit of 2000 nm. As in previous studies, Poisson's ratio was assumed to be 0.3 for the bone specimens [10], [14], [15]. A total of 10 to 15 indents per specimen were performed and for each indent, average modulus and hardness values were obtained between indentation depths of 800-1600nm (a range over which these measurements were approximately constant). The indentation test was performed in four separate, pre-defined clusters and located in a different region of lamellar bone for each specimen. The microstructure of bone was described by using optical microscope for each indent site. There are two microstructural groups for every indent sites: osteonal bone and interstitial lamellar bone. There were also excluded from the data set that associated with any indents which were found to be in contact with void spaces such as osteocyte lacunae, resorption spaces and Haversian canals. The nanoindentation tests were conducted at different loads for every specimen. A total of 40 indents were included in this study. Approximately, half of the indents were in the specimens of interstitial region, while the other half were in those of osteonal region. The elastic modulus and hardness of bone were determined by applying the method adopted by Oliver and Pharr [16].

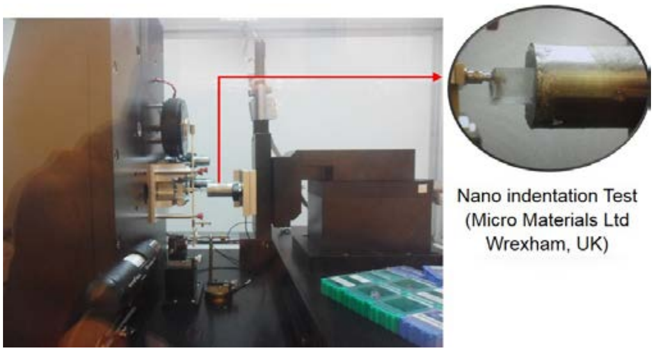

Figure 2: Experimental setup for nanoindentation test

3. RESULTS

Figure 3 depicts the typical load-depth curves for interstitial and osteon of OI bone under nanoindentation test. There are 20 indents representing the plots for each graph. In each indentation, the same experiment procedure was used. The load-depth curve in interstitial OI bone shows increases compared to osteon OI bone. For (a) interstitial bone, the maximum load was 70 mN, whereas for (b) osteon bone, was 40 mN at the maximum depth.

Based on the load-depth curve, the hardness (H) and reduced modulus (E_r) values were calculated and plotted as shown in Figures 4 and 5 respectively. The hardness is related to the bone strength. From the graph in Figure 3, the distribution of the hardness shows in different pattern between interstitial and osteon of OI bone. Plots distribution of hardness for interstitial shows quite consistent with maximum value of 0.97 GPa, whereas plots for hardness of osteon shows inconsistent pattern with 0.51 GPa of maximum value. The plots distribution of reduced modulus for interstitial and osteon bone are shown in Figure 5. The results of reduced modulus for each indent of interstitial and osteon bone are quite scattered. The highest value was found at 29.98 GPa for interstitial, and 14.85 GPa for osteon bone.

On the other hand, Figure 6 (b) show the mean and standard deviation of hardness that was obtained as 0.6 ± 0.11 GPa for interstitial bone and 0.44± 0.06 GPa for osteon. Whereas Figure 6(b) depicts the the mean and standard deviation of reduced modulus that was obtained as 19.0 ± 4.23 GPa for interstitial bone and 12.46 ± 1.49 GPa for osteon. Obviously, the results indicate that the hardness and reduced modulus of OI bone at interstitial are higher than at osteon.

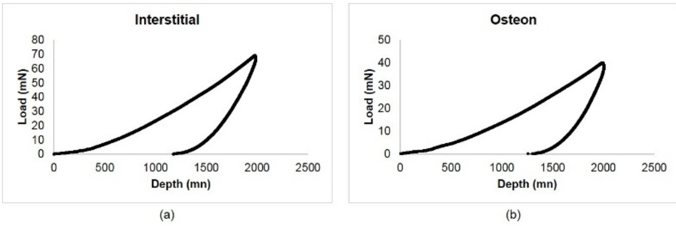

Figure 3: Load-depth curve for (a) interstitial, and (b) osteon of OI bone.

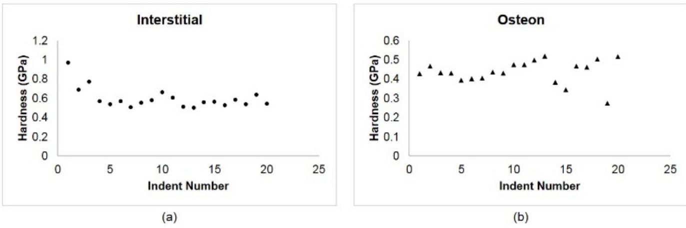

Figure 4: Plots of hardness for each indentation at (a) interstitial and, (b) osteon of OI bone.

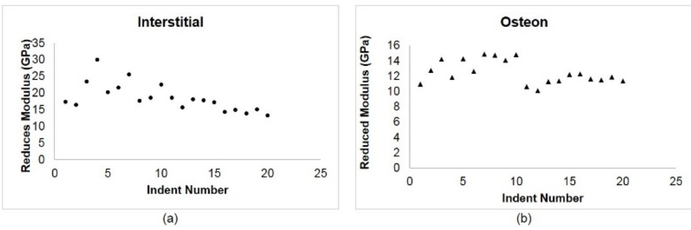

Figure 5: Plots of reduced modulus for each indentation at a) interstitial and, b) osteon of OI bone.

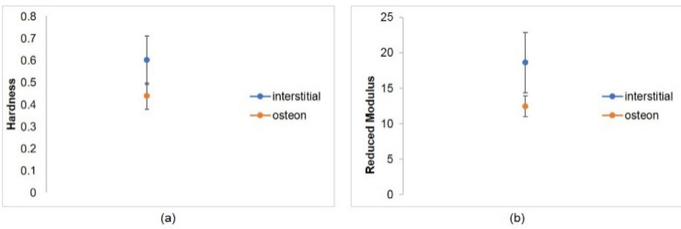

Figure 6: Mean and standard deviation of nanoindentation results for (a) hardness and, (b) reduced modulus.

4. DISCUSSION

This study aims to determine the mechanical properties of hardness and reduced modulus for type III OI bone. The OI bone has been divided into two sections which were interstitial and osteon bone. The mechanical properties have been analysed were hardness and reduced modulus for both section of bones. In this work, reported that the values of load-depth curve show difference between interstitial and osteon bone. According to this result, the observation may imply that because osteonal bone undergoes greater creep-deformation than interstitial bone. In addition, the toughness in osteon may be tougher than interstitial bone due to cyclic indentation loading seen mainly in osteon accumulates in the organic matrix through creep-related mechanism. The creep was correlated with fracture toughness between both bones. Several authors [17], [18] justified that interstitial bone has lower indentation load-depth which indicate that the extent material softening is lower than osteon bone. The softening bone often cause stress concentration and microcrack propagation in the matrix. Also, previous studies have reported that the softened of interstitial bone interface could result from higher mineralization and impaired organic matrix containing posttranslational modifications [19]. Indeed, several studies reported that microcracks often occurred at the osteonal-interstitial bone interfaces [18], [20].

In order to measure the bone strength, the hardness response has been tested in this study. According to our result, the hardness response was found to be difference between interstitial and osteon bone. The hardness in interstitial shows increases than osteon bone. This is because of the behaviour of each bone due to the maturation state of the tested. This means that the interstitial had suffered many loading too which causes the mineral content decreases within the structure between osteon [21]. By contrast, values of hardness in osteon bone were found to be lower than values of interstitial bone. This is due to bone tissue behaviour is viscoelastic in nature. In addition, based on result in Figure 5, mean and standard deviation for hardness was 0.6 ± 0.11 GPa for interstitial bone, while 0.44± 0.06 GPa for osteon. Previously, a few studies in local mechanical properties in human OI bones by nanoindentation, which also find the result of hardness consistent with our study. It is worth noting that the range of hardness within 0.3-0.9 GPa for severe OI. However, Fan et al. [22] measured a mean value of 19.67 GPa for the human cortical bone of OI type III, and Albert et al. [6], [20] found a mean value of 16.3 GPa for the same OI type. Nevertheless, neither of these studies compared their results to a human control group.

Calculation of reduced modulus assumes local isotropy of the mechanical properties of the bone specimen for interstitial and osteon bone. Results in Figure 4 present the distribution of reduced modulus for interstitial and osteon of OI bone. As can be seen, increased reduced modulus with increasing indent more in interstitial than osteon until it reached a maximum value at 29.98 GPa for interstitial and 14.85 GPa for osteon bone. This is believed to occur due to bone microstructure and therefore the result was higher in interstitial lamellar regions than osteonal regions. This is in agreement with the results reported by another researcher in nanoindentation studies of bovine [23] and adult human bones [13]. The difference between the regions due to the differences of degree mineralization. It seems interstitial region tend to be more mineralized than osteonal region (Eschberger 1986). In addition, as shown in Figure 6, mean and standard deviation for all indents for interstitial region was 19.0 ± 4.23 GPa, while 12.46 ± 1.49 for osteon respectively. This result obtained agreed with previous work carried out by [24], who reported that, the reduced modulus for interstitial 19-22 GPa.

The current study presents the mechanical characterization of reduced modulus and hardness of OI type III bone tissue. Although this study did not include a normal control group, the result can be compared against those of other previously published studies to get a sense of how hardness and reduced modulus compares between OI phenotypes and normal bone. It can be seen, reduced modulus and hardness slightly increased at interstitial region rather than osteonal bone. The structural behaviour of whole bone is dependent not only on material properties but on geometry as well. In the present study, of all the results presented, there is a limitation on the mechanical properties analysis. However, present study only conducted the analysis on reduced modulus and hardness. This show that the result was very limited and can be disputed. This study can be enhancing by doing more analysis on other mechanical properties such as young's modulus, tensile strength and also the difference directions which are in longitudinal and transverse. In addition, it is warranted to be included in the future discussions which are the correlation between reduced modulus and hardness for all the directions.

5. CONCLUSIONS

Results of the present study indicate that the mechanical properties by nanoindentation test were affected by OI severity. The reduced modulus and hardness are higher in interstitial bone region than osteonal bone region. Based on the current findings, it would be ideal in future studies aimed at characterizing the mechanical properties of OI bone considered each phenotype separately. Further work is recommended to determine how other material properties such as bone material strength and toughness, are affected by OI bone severity. The knowledge of these mechanical properties for any bone material in OI bone could contribute to the ability to develop models to assist in predicting fracture risk.

CONFLICT OF INTEREST

The authors declare that they do not have any conflict of interest which could affect the outcome of this study.

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