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Measuring of Specific Bone Alkaline phosphatase (BAP) Bone Remodeling biomarker for Post-COVID Iraqi Patient

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Abstract

Background: There are several diseases in the body following recovery from COVID-19 infection because this virus operates on human genes in various types of peripheral tissue in the human body. It penetrates host cells via Angiotensin-converting enzyme-2 receptors and may have effects on bone remodeling, leading to osteopenia or osteoporosis, which are characterized by low bone mineral density, resulting in diminished bone strength. Bone Alkaline Phpsphatase is an enzyme released into the bloodstream as a soluble homodimer after being cleaved by a phospholipase and can be utilized as a biomarker of bone development.

Objective: This research was designed to investigate the alteration of bone homeostasis balance in Iraqi post-COVID-19 infection patients.

Cases and Methods: This is a case control study. The study has received approval from the ethical committee at the Faculty of Medicine, Baghdad University, established on November 20, 2021– March 2, 2022. A hundred and thirty individuals were enrolled in this study. The subjects were divided into two groups; the first group (80) post-COVID-19 infection patients and the second group (50) non-COVID-19 individuals. Also, measuring markers like serum Angiotensin-converting enzyme-2 and Bone Alkaline Phosphatase by using the ELISA technique. The bone mineral density was measured by a DEXA scan.

Results: This study found that there is an effect of coronavirus infection on the bone strength measured by the mean ± SD Bone Alkaline Phosphatase level, which was found to be highly significant in the serum of post-COVID-19 patients when compared with non-COVID-19 individuals (P-value = 0.001), but the mean ± SD of Angiotensin-converting enzyme-2 level was statistically non-significant between the two groups (P-value = 0.13). who had recovered from a coronavirus infection for 3 months or more. Also, the bone mineral density of Post COVID-19 patients that was measured by DEXA scan had a highly significant T-score% when compared between the two groups.

Conclusions: This research found that COVID-19 has an impact on the bone remodeling process, leading to osteopenia or osteoporosis, which may be identified by checking the blood levels of the bone biomarker BAP and the bone mineral density (by DEXA scan) at least three months following coronavirus recovery. This investigation also discovered that some of the individuals had osteopenia rather than osteoporosis.

Keywords: ACE-2, BAP, COVID-19, DEXA scan , Osteoporosis .

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Introduction:

After the 2019 coronavirus disease (COVID-19) pandemic emerged in Wuhan, China, in December 2019, the World Health Organization (WHO) declared it a global health emergency [1]. The structural proteins of the viral envelope are spike proteins made of a trimeric glycoprotein that protrudes like a crown from the host cell, just like those of other coronaviruses [2] .When COVID-19 binds to ACE-2 (the angiotensin converting enzyme-2) by its S spike into host cells, , COVID-19 can enter and infect cells [3] . Bone growth is the consequence of modeling, which involves regenerating bone substance and modifying bone size and shape, whereas bone health is maintained through remodeling, which involves replacing old bone with new bone. The adult skeleton's structural integrity and strength are continuously preserved by the cycle of bone remodeling. The basic multicellular unit (BMU) of bone remodeling is composed of osteoclasts and osteoblasts, and osteocytes regulate their activity [4]. There is a continuous remodeling cycle. The remodeling cycle's stages might range in length. Before the new bone structure unit is fully produced, formation can continue for up to 4 months during the reversal phase, 2 weeks for resorption, and 4 or 5 weeks for the reversal phase[5] .The four different types of bone cells—osteocytes, osteoblasts, bone lining cells, and osteoclasts—are in charge of forming and shaping bone [6] .Bone lining cells, although the majority of the trabecular bone surface is covered by

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bone lining cells, these cells probably play a significant role in biochemical regulation [7] . Over 200 bone pieces are formed by osteoblasts, which account for about 15% of the overall mass of the human body. Osteoblasts produce considerable amounts of collagenous and noncollagenous proteins in the bone matrix that act as a scaffold for matrix mineralization by permitting the deposition of calcium phosphate in the form of hydroxyapatite[8] . Osteoclasts, which are in charge of bone resorption and arise from myeloid progenitor or osteal macrophage hematopoietic stem cells (HSCs), are well defined and identifiable in bone marrow. When activated, monocytes in the peripheral circulation go to a specific spot in the bones, where they merge to form mature multinucleated osteoclasts [9] . Osteoclasts are important in the remodeling of soft and hard calluses during bone development and fracture repair [10] . Osteocytes, one of the most persistent cell types in the human body, account for 95% of the total number of cells in bone tissue and have a half-life of 25 years on average. Osteoblasts and osteoclasts, on the other hand, have a short lifespan and account for just around 5% of all bone cells [11] Aside from pulmonary damage and sudden respiratory collapse, the virus damages a variety of organs, including the heart, kidneys, and digestive tract. After a long-term infection, the virus spreads through the bloodstream to other organs, causing infection and the destruction of the host's cells. High coronavirus infection affects the liver, bile ducts, and pancreas due to enhanced ACE2 expression [12] . COVID19-induced inflammation has been found to have a negative influence on the musculoskeletal system through a variety of different routes. SARS-CoV-2 penetrates cells of various tissue types via ACE2, including "smooth muscle," synovial tissue, and cartilage. ACE2 plays a number of activities, including anti-inflammatory actions and bone resorption inhibition, which has been linked to skeletal muscle injury and illness [13] . Coronavirus infection may have an influence on bone remodeling [14].Specific Bone Alkaline phosphatases BAP are membrane-bound glycoproteins that catalyze the hydrolysis of phosphate monoesters such as inorganic pyrophosphate (PPi). BAP is one of four isozymes currently known in humans, depending on the place of tissue expression [15] . BAP is a homodimer that is linked to osteoblast and matrix vesicle membranes (MVs). They share the same amino acid structure but differ only in post-translational modifications. After being broken by a phospholipase, BAP enters the bloodstream as a soluble (anchor-free) homodimer and can be employed as a biomarker of bone development .Tissue-nonspecific Alkaline phosphatases TNALP isoforms from bone and liver are the most prevalent in serum from healthy human adults, with an about 1:1 ratio [16] .The BAP found on the surface of osteoblasts and reflecting their biosynthetic activity has been shown to be a sensitive and reliable indicator of bone metabolism [17] . Osteopenia and osteoporosis are skeletal conditions characterized by decreased bone strength and an increased risk of fracture. The definition of bone strength includes bone mineral density (BMD). Fragility fractures are most common in the spine, hip, and distal forearm. Fragility fractures are a substantial contributor to disability and decreased life expectancy [18] . The current study's goal was to determine the bone homeostasis balance in Iraqi post-COVID-19 patients and to identify the effect of the viral infection on both osteoclastogenesis and osteoblastogenesis by detecting the change in osteoprotegerin levels in their blood.

Subjects and Methods:

This research is case control study . established on (November 20, 2021–March 2, 2022). A hundred and thirty individuals were enrolled in this study. They were divided into two groups. The first group included 80 patients (they have been diagnosed with COVID-19 by PCR or chest CT scan and have been recovered from coronavirus infection for 3 months or more. The second group included 50 subjects that haven't been infected with the coronavirus. All the individuals were taken from Al Yarmouk Teaching Hospital and the Shifa Center City of Medicine Hospital. Their age range was between 18 to 45 years old for women and 18 to 60 years old for men. The serum Angiotensin-converting enzyme-2 (ACE-2) was measured using a commercial enzyme-linked immunosorbent assay ELISA technique provided by the Human ACE2 ELISA Kit, made in China. The serum Bone Alkaline Phosphatase (BAP) levels were also measured by using the ELISA technique provided by the Human Bone Alkaline Phosphatase (BAP) ELISA Kit, made in China. The bone mineral density (BMD) was measured by dual-energy Xi-ray absorptiometry (DEXA scan). The World Health Organization (WHO) has established standards for the diagnosis of osteoporosis based on the accuracy and repeatability of DEXA scans [19]. T-scores are used in the diagnosis of osteoporosis normal BMD is defined as a T-score of 1.0, Lowi bone mass or osteopenia is defined as a T-score between -1.0 and -2.5, whereas T-scores over -2.5 indicate osteoporosis [20].Statistical analysis : The datae were analyzed using the Statistical" Package for Social Sciences (SPSS)i version" 26 . And the data were presented as mean , standard deviation (SD), The correlation between continuous variables was evaluated using Pearson's correlation test (r), in accordance. Statistical significance was defined as a P-value of 0.05 or less [21] .

Results

In the current study The study participants' age ranged between 18 to 45 years old for women and 18 to 60 years old for men. (Table 1) .

Table 1 : Comparison between study groups by age and BMI

Characteristics	The Groups		P – Value
	COVID-19 patients Mean ± SD	Non-COVID-19 individuals Mean ± SD	
Age (Years)	31.4 ± 8.3	30.7 ± 9.0	0.658
BMI (kg/m2)	28.35 ± 4.9	28.07 ± 4.1	0.731

In the comparison between the two groups, there were no significant differences in mean and standard division ±SD for the age, ratio and (BMI) between the two groups . Comparison between two groups of some biochemical markers and DEXA score% as shown in (Table2)

Table2 : Comparison in biological markers and DEXA score by previous COVID-19 infection

Parameters	The group		P - Value
	COVID-19 patients Mean ± SD	Non-COVID-19 individuals Mean ± SD	
ACE2 (pg/ml)	173.25 ± 36.7	185.74 ± 50.0	0.13
Bone ALP (ng/ml)	140.98 ± 17.9	98.01 ± 11.7	0.001**
DEXA score (%)	- 0.43 ± 0.94	0.45 ± 0.64	0.001**

**** statistically highly significant .**

There were significant differences in the mean ± SD of BAP level in serum between the two groups [post COVID-19 (140.98 ± 17.9), Non COVID-19 individuals (98.01 ± 11.7) ng/ml]with a P-value of ≤ 0.001, (P-value≥0.05 considered statistically significant) , and there were no significant differences in the mean ± SD of ACE-2 level between the two groups[post COVID-19 (173.25 ± 36.7) , Non COVID-19 individuals (185.74 ± 50.0) ,] with (P-value ≤ 0.13) as shown in (Table 2) .

Table 3: Correlation between biological parameters

Parameter		ACE2	BAP	DEXA Score
ACE2	r	-	- 0.003	- 0.98
	P-Value	-	0.974	0.266
Bone ALP	r	- 0.003	-	- 0.558
	P-Value	0.974	-	0.001**
DEXA score	r	- 0.98	- 0.558	-
	P-Value	0.266	0.001**	-

**** statistically highly significant .**

Statistically significant strong positive correlation was detected between bone ALP and DEXA score (r=- 0.558) with (P-value ≤0.001) as shown in (Table 3) and figures (1).

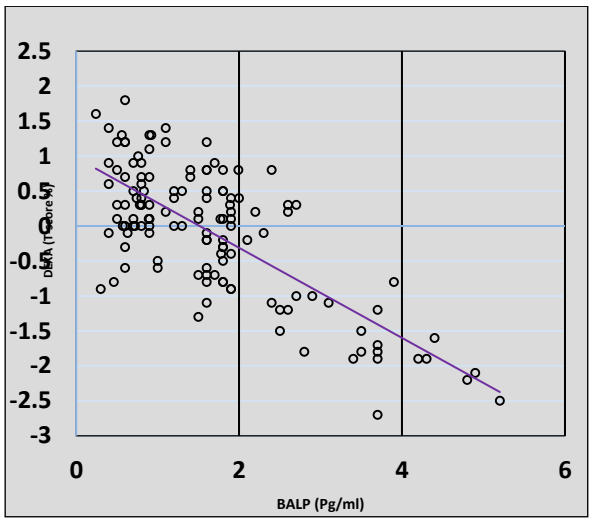

Figure 1: Correlation between bone ALP and DEXA score

Discussion:

Bone remodeling is a bone turnover mechanism that involves the destruction of old bone cells by osteoclasts and the formation of new bone cells by osteoblasts [21] . whereas the functioning of ACE-2 is related to the "renin-angiotensin-aldosterone,system (RAAS), which functions as a coronavirus entrance receptor. When a coronavirus connects to several types of cells, it can cause damage to multiple organs in the host body by activating the immune system like skeletal system [22] .IL-2, IL-7, IL-10, tumor necrosis factor (TNF), osteoprotgerin (OPG), and granulocyte colony stimulating factor are just a few of the proinflammatory cytokines that are produced in abundance during this storm as a result of the immune system's hyperactive response to the SARS-CoV2 virus [23] .One of the ALP enzyme isomers that is detectable in the bloodstream that is formed from bone cells is the specific bone alkaline phosphatase enzyme (BAP). ALP and BALP produced by bone cells account for about 50% of serum ALP. Think of a bone-formation biomarker that plays a role in the mechanism of bone remodeling. An indicator of osteoblastic activity, the measurement of BAP is used to help manage osteoporosis [24] . In this study, there was a highly significant difference in the level of BAP after recovery from COVID-19 infection with a P-value of 0.001. It was found in the high level in the serum of patients with post COVID-19 infection and with low level in people who are not infected with COVID-19 and that may have an effect on bone remodeling mechanism. BAP has been demonstrated to be a sensitive and reliable biomarker of bone metabolism in other studies. Additionally, it serves as a trustworthy biochemical marker of bone growth in osteoporosis and other bone problems. According to numerous studies, measuring the level of BAP in the serum may be a more accurate way to gauge bone density than using any other markers [25] . BMD and serum BAP are inversely associated. Another different study discovered a negative correlation

between serum BAP and BMD [26]. The inorganic pyrophosphate and mineral formation in the bone can be decreased by a specific enzyme known as BALP. The most common non-collagen protein that modifies the mineral matrix deposition throughout the mineralization process is BALP [27] .Other studies found that coronavirus infection affects the skeletal system may have an effect on bone remodeling , and that ACE-2 contributes to the production of osteoblasts and osteoclasts by inhibiting bone resorption in the MasR pathway [28] . Inflammatory cytokines like IL-1, IL-6, TNF-, G-CSF, IP-10, MCP-1, and MIP-1 are found to be elevated during coronavirus infection and have an impact on the osteoclastogenic pathway by decreasing osteoblast and OPG levels and increasing osteoclast hypoxia, which affects bone activity [29] . Because high-dose glucocorticoids, the typical treatment for COVID-19 hospitalized patients, are known to decrease bone mineral density (BMD) and increase fracture risk in a dose- and duration-dependent manner, the current COVID-19 medications may have a negative impact on bone health [30] . During the COVID-19 pandemic, estrogens can also activate the local immune system, which may be a key factor in explaining why women experience a lower prevalence of COVID-19 than men [31] . Lack of estrogen is another possible cause of osteoporosis in older women who have undergone menopause, as it speeds up the loss of cortical bone and prevents calcium from being absorbed and used [32] .

Conclusions:

This study found that COVID-19 has an impact on the bone remodeling process, leading to osteopenia or osteoporosis, which may be identified by checking the blood level of the bone biomarker BAP and the bone mineral density (by DEXA scan) after at least three months of coronavirus recovery. This investigation also found that some of the individuals had osteopenia rather than osteoporosis .

Authors' Contributions:

Islam S Al-Azzawi : student
Nawar S Mohammed : 1st supervisor
Nizar Abdulateef Jassim :2nd Supervisor

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قياس مستوى أنزيم الفوسفاتيزالقلوي العظمي احد علامات الحيوية لاعادة تشكيل العظام للمرضى العراقين بعد الاصابة ب COVID-19

الكيمياوي االاختصاص إسلام سعد العزاوي *طالب ماجستير فرع الكيمياء سريرية /كلية الطب / جامعة بغداد.
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الاستاذ الدكتور نزار عبد اللطيف جاسم فرع الباطنية /كلية الطب / جامعة بغداد.**

الخلاصة:
الخلفية: هناك العديد من الأمراض في الجسم بعد الشفاء من عدوى كورونا لأن هذا الفيروس يعمل على الجينات البشرية في أنواع مختلفة من الأنسجة الطرفية في جسم الإنسان. يخترق الخلايا المضيفة عبر مستقبلات الإنزيم المحول للأنجيوتنسين 2 , وقد يكون له تأثيرات على إعادة تشكيل العظام ، مما يؤدي إلى هشاشة العظام ، والتي تتميز بانخفاض كثافة المعادن في العظام مما يؤدي إلى انخفاض قوة العظام. يتم تحرير أنزيم الفوسفاتيزالقلوي العظمي في مجرى الدم القابل للذوبان بعد انشقاقه بواسطة انزيم فوسفوليباز ويمكن استخدامه كمؤشر حيوي لنمو العظام .
الهدف: تم تصميم هذا البحث للتحقيق في تغيير توازن التوازن العظمي في مرضى عدوى ما بعد الاصابة بفايروس كورونا للمرضى العراقين .
طريقة العمل و العينات: هذه دراسة مقطعية. حصلت الدراسة على موافقة لجنة الأخلاقيات في كلية الطب جامعة بغداد ، والتي تأسست في 20 تشرين الثاني (نوفمبر) 2021 - 2 آذار (مارس) 2022. وقد تم تسجيل جميع المواد الـ 130 في هذه الدراسة. تم تقسيم الموضوعات إلى مجموعتين ؛ المجموعة الأولى (80) مرضى مصابة سابقا بفايروس كورونا والمجموعة الثانية (50) لناس لم تصاب كورونا سابقا, يتم قياس تحاليل مثل مصل مستوى الإنزيم المحول للأنجيوتنسين-2 و أنزيم الفوسفاتيزالقلوي العظمي باستخدام تقنية الاليزا . تم قياس كثافة المعادن في العظام بواسطة مسح الدكسا .
النتائج : وجدت هذه الدراسة أن هناك تأثيرًا لعدوى الفيروس التاجي على قوة العظام من خلال متوسط الانحراف أنزيم الفوسفاتيزالقلوي العظمي , والذي وجد أنه يوجد فرق في المرضى المصابقة (P= 0.31)لكن متوسط الانحراف لانزيم المحول للانجيوتنسين 2 لم يتغير بين المجموعتين حيثقيمة الP)بفايروس كورونا سابقا و الناس التي لم تصاب بكورونا و عند قياس قيمة ال(0.001>
عالية مهمة عند المقارنة بين المجموعتين . T. و كذلك كانت الكثافة المعدنية للعظام لمرضى ما بعد الاصابة بكورونا التي تم قياسها بجهاز الدكسا تحتوي على درجة
الاستنتاجات: وجد هذا البحث أن فايرو كورونا له تأثير على عملية إعادة تشكيل العظام ، مما يؤدي إلى هشاشة العظام أو هشاشة العظام ، والتي يمكن تحديدها عن طريق فحص مستويات الدم للعلامة الحيوية للعظام أنزيم الفوسفاتيزالقلوي العظمي و كذلك قياس كثافة المعادن في العظام (عن طريق مسح الدكسا) على الأقل ثلاثة أشهر بعد الشفاء من فيروس كورونا. اكتشف هذا التحقيق أيضًا أن بعض الأفراد يعانون من هشاشة العظام .
الكلمات الرئيسية : كورونا-19 , إنزيم المحول للأنجيوتنسين 2 , أنزيم الفوسفاتيزالقلوي العظمي , هشاشة العظام , جهاز الدكسا .