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Determination of Osteocalcin, Osteopontin,Osteonectin Hormones and Interleukins levels in Postmenopausal Women Osteoarthritis in Kirkuk City

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Abstract

The current study included the collection of 80 samples, 50 samples from patients with osteoporosis and 30 samples from non-affected women (control group) whose ages ranged between (55-80) years. The samples were gathered from outpatient medical clinics in Kirkuk, and then blood was drawn from healthy and sick persons and separated using a centrifuge.

The centrifuge was used to assess the amounts of C-reactive protein, osteocalcin, osteopontin, ,osteonectin Interleukin-IL-6 and IL1.The current study found a substantial increase (P ≤0.05) in the level of hs-CRP in patients with osteoporosis, as it was (0.917 ±0.368) ng /ml, while the control group (healthy women) was (0.588 ±0.132) ng / ml, and a significant drop (P≤0.05) in the concentration of Osteocalcin.as it was (0.921±0.098) ng/ml in osteoporosis patients, while the control group (healthy women) was (1.8010.199) ng/ml, and a significant increase (P≤ 0.05) in the level of Osteopontin was (2.789 0.891) ng/ml in osteoporosis patients, while the control group (healthy women) was (0.987 ±0.296) ng/ml, And a substantial increase (P ≤0.05) in the level of Osteonectin, which was (2.8960.567) ng / ml in osteoporosis patients.While the control group (healthy women) was (1.409±0.164) ng/ml, and a substantial rise (P≤ 0.05) in interleukin-6 concentration. It was (20.53 ±26.536) ng / ml in osteoporosis patients, whereas the control group (healthy women) was (10.23±01.987) ng / ml, and there was a significant rise (P ≤0.05) in the interleukin-1β among the patients (16.044±1.252) ng / ml. Female osteoporosis patients served as the control group (healthy women), while it was (8.7871.980) ng/ml in the healthy women group.

Key words / Osetoprosis, Osteocalcin , Osteopontin, , Osteonectin, Interleukins

Introduction

Osteoporosis is a worldwide public health problem affecting millions of people andis more common in postmenopausal women. Osteoporosis symptoms include decreased bone density and an increased risk of fracture owing to bone structural deterioration. Fractures increase morbidity and mortality considerably (Beomchang, et al, 2021).As a result, drug therapy containing bone-forming and bone-strengthening antibiotics such as bisphosphonate antibiotics, , estrogen receptor modulators of selectivity (SERMs),and anti-receptor activator of antibody NF-B (RANK) (RANKL) (Denosumab)can minimize osteoporotic fractures (Langdahl, 2021).

Age-related osteoporosis is caused by continuing bone disintegration, which is accompanied by a loss of sex hormones. Furthermore, decreased estrogen production causes bone loss in postmenopausal women. Sex hormone-binding globulin decreasesestrogen activity andtestosterone with age, which may contribute to lower BMD (Hunter DJ, 2000, Jeremiah MP, 2015). Osteoporosis has been connected to a variety of biochemical factors, including osteocalcin (OC), a small non-collagenous bone protein found mostly in bones and generated by osteoblasts. It is a sensitive marker of bone development. Acetocalcin is released into the bloodstream in trace amounts and contributes in lipid and glucose metabolism (S. B. Oh, 2019).

Osteopontin is a phosphoprotein that controls homeostasisand bone metabolism is an important role in bone mass. It also performs biological functions like as reproduction, migration, and attachment to different bone-associated cells, including stem cells. Mesenchymal cells in bone marrow, hematopoietic stem cells, osteoclasts, and osteoblasts are all implicated in the rheumatoid arthritis anddevelopment of bone diseases such osteoporosis (Jinyan, 2020).It is quite fond of type I collagen and hydroxyapatite (Bradshaw, 2012)

It was also discovered that osteoporosis has a relationship with interleukins, specifically interleukin (IL-6), which is participatein a group of age-related diseases, including osteoporosis, whose time courseand initiation are influenced by inflammatory

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cytokines, as IL-6 levels were found to be elevated during the ongoing processes menopauseand of aging. This is seen by the activation of osteoclasts and bone resorption (Khosla S., 2002). Koshihara., 2002 In addition, interleukin IL-1, a pro-inflammatory cytokine that contributes to bone degeneration in a number of diseases, including osteoporosis and rheumatoid arthritis (Rongdong, 2021).

Material and Methods

The study was conducted on 80 blood serum samples from postmenopausal women, 50 samples from patients with osteoporosis, 30 samples from healthy women (control group) and their ages ranged between (55-85) years. Samples of patients were collected from outpatient medical clinics in Kirkuk for the period from 20 /2/2022 until 04/20/2022 The level of Osteocalcin, Osteopontin, and Osteonectin was measured, as well as the level of Interleukin-IL-6, and (IL1B).

Determination of,hs-CRP,Osteonectin ,Oteopontin , Oteocalcin,IL-6, and IL1Blevels in the blood sample

Serum hs,CRP, osteonectin, osteopontin, osteocalcin,IL-6, and IL1B levels were evaluated using a kit assayed to the specifications of the manufacturer (SunLong Biotech Co.,LTD, Cat. No. SL2458Hu, SL276Hu and SL274Hu, China).

Statistical Analysis

The statistical program SPSS was used to analyze the results obtained if the arithmetic means and the standard deviation value of the data under study were used, and the Analyzed variables were compared using a T-test, between the two groups of patients and control at the level of probability ($P \leq 0.05$).

Results and discussion

Measuring the levels of the studied variables for the samples under study:

Table 1.Shows the mean ± standard deviation of the Variable for the samples under study.

Groups / Variables	Mean ± SD		P-Value
	Control n=30	Patients n=50	
CRP(ng/ml)	0.588±0.132	0.917±0.368	P≤ 0.05
Osteocalcin(ng/ml)	1.801±0.199	0.921±0.098	P≤ 0.05
Osteopontin (ng/ml)	0.987 ± 0.296	2.789 ± 0.891	P≤ 0.05
Osteonectin (ng/ml)	1.409±0.164	2.896±0.567	P≤ 0.05
IL-6 (ng/ml)	10.230±1.987	20.532±6.536	P≤ 0.05
(IL1B) (ng/ml)	8.787±1.980	16.044±1.252	P≤ 0.05
P<0.05 = *			

Estimation of the level of CRP in the blood serum

Table (1) shows the mean ± standard deviation of the level of hs-CRP, which was (0.917±0.3 ng/ml in patients with osteoporosis, while the control group (healthy women) was (0.588±0.132) ng/ml.

The results demonstrated a substantial rise in the amount of Osteocalcin in the serum of osteoporosis patients compared to healthy women at the level of probability P≤ 0.05, as shown in Figure (1).

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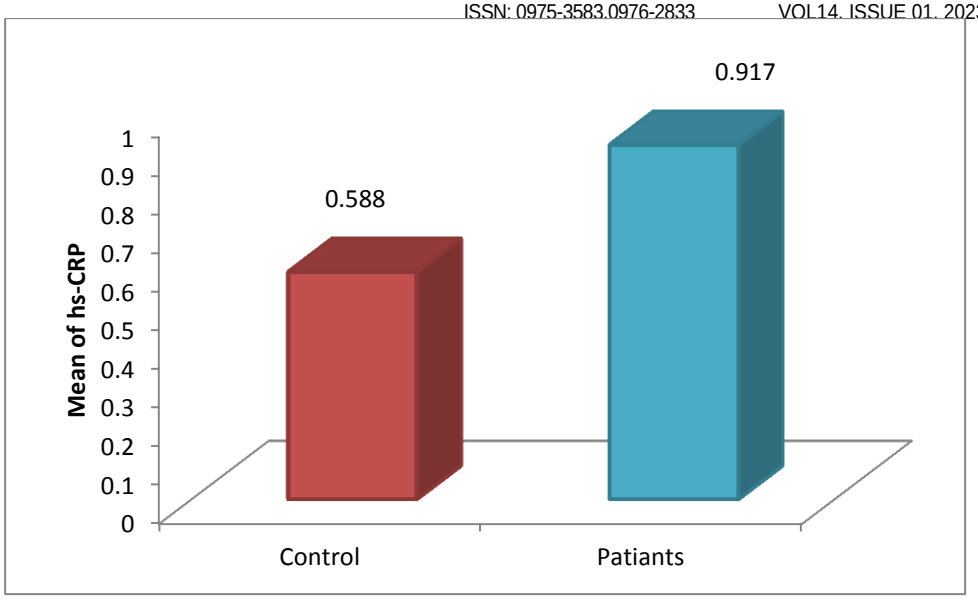

Figure (2): - hs-CRP level in the blood serum of samples

The study's findings corresponded with those of (Jeedigunta1. 2020), who found an increase in C-reactive protein levels in the blood serum of osteoporotic women.

It has been revealed through study that there is a correlation between low bone density, OP and CRP, as well as a link between weak or thin bones andhigh CRP (Jensen et al., 2005; Palmowski et al., 2022). On the other hand, it has been demonstrated that inflammatory processes regulate many cytokines, such as TNF- and IL-1, IL-6, which significantly influence hepatic CRP formation and bone resorption (Weinhold., 1997, Yoshida., 2002).

Estimation of the level of Osteocalcin in the blood serum

Table (1) shows the mean ± standard deviation of the level of Osteocalcin, which was (0.921 ± 0.098) ng/ml in patients with osteoporosis, while the control group (healthy women) was (1.801 ± 0.199) ng/ml.

The results demonstrated a substantial drop in the amount of Osteocalcin in the serum of osteoporosis patients compared to healthy women at the level of probability P ≤0.05, as shown in Figure (2).

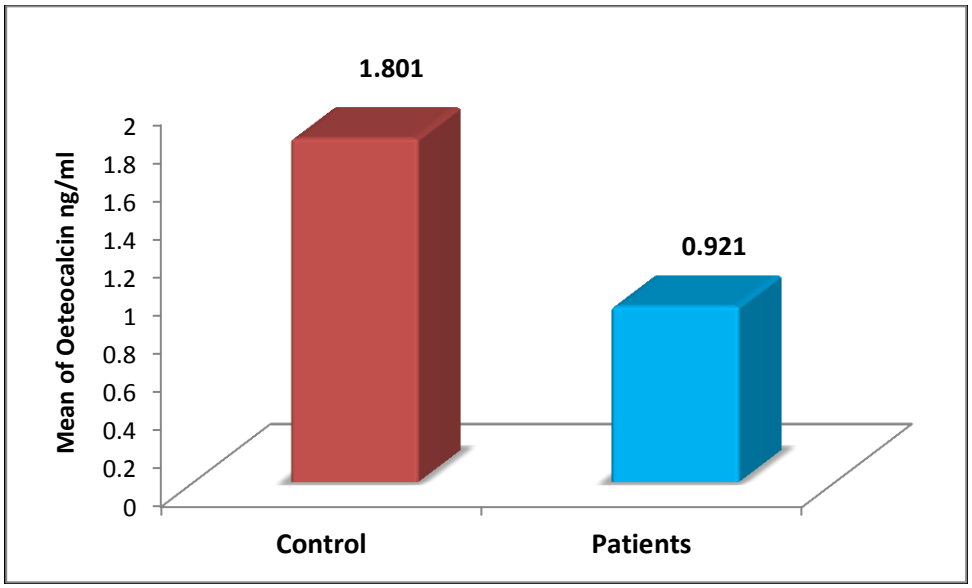

Figure (2): - Osteocalcin level in the blood serum of samples

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Estimation of the level of osteopontin hormone in the blood serum

Table (1) shows the mean ± standard deviation of osteopontin level, which was (2.789 ± 0.891) ng / ml in patients with osteoporosis, while the control group (healthy women) was (0.987 ± 0.296) ng / ml.

The results demonstrated a substantial rise in the level of Osteopontin in the serum of osteoporosis patients compared to healthy women at the level of probability P 0.05, as shown in Figure (3).

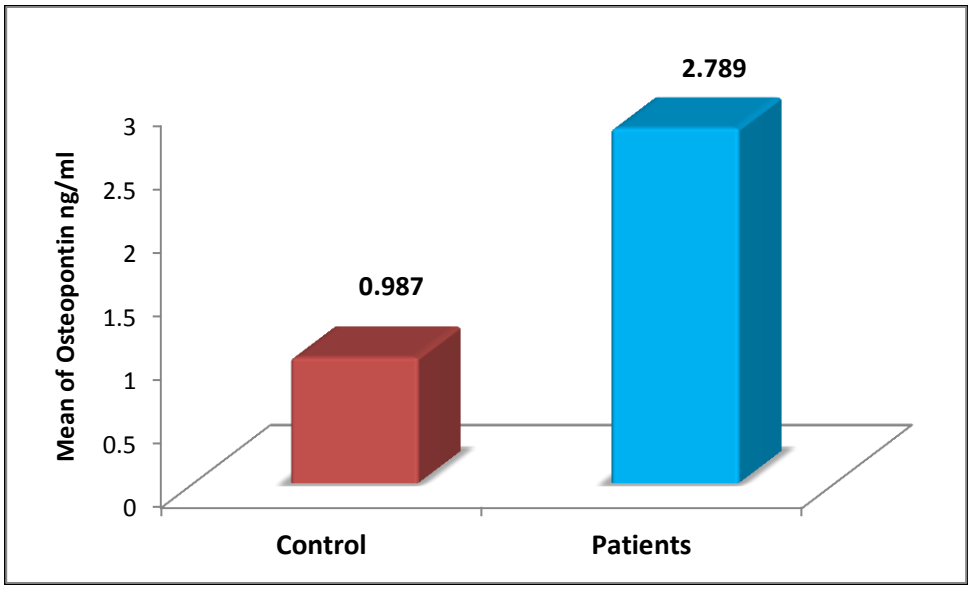

Figure (3): - Osteopontin level in the blood serum of samples

Estimation of the level of the hormone Osteonectin in the blood serum

Table (1) shows the mean ± standard deviation of the level of Osteonectin, which was (2.896 ± 0.567) ng / ml in patients with osteoporosis, while the control group (healthy women) was (1.409 ± 0.164) ng / ml.

At a probability level of P ≤0.05, the results demonstrated a substantial rise in the level of Osteonectin in the serum of osteoporosis patients compared to healthy women as a control group, as shown in Figure (4).

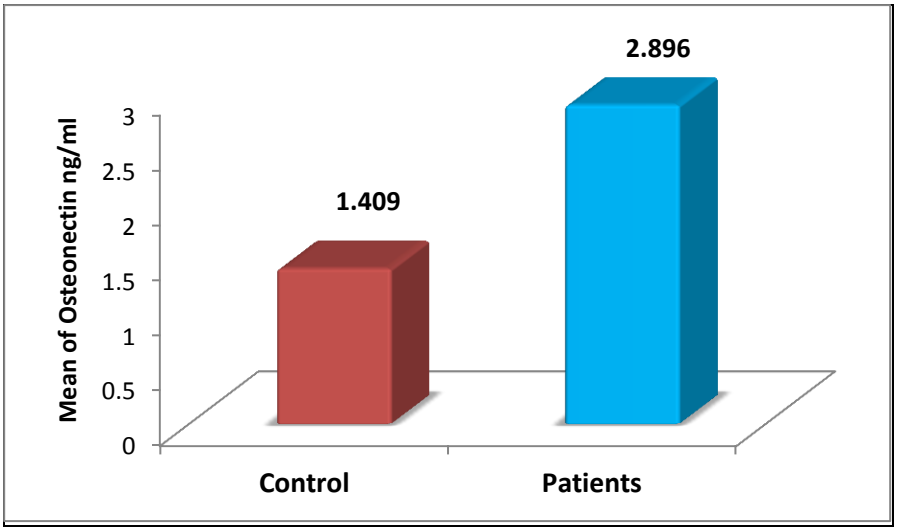

Figure (4): Osteonectin level in the blood serum of samples

The study's findings corresponded with those of (Ali B. Roomi, et al, 2022) and (Zhongyu*et al*., 2019), who found a significant drop in the level of Osteocalcin in their study. Significantly in people with osteoporosis, decreased levels of Osteocalcin may be related to a lack of estrogen produced by ovarian failureOsteocalcin is a small non-collagen protein found

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mostly in bone and synthesized by osteoblasts. Because it is primarily absorbed into the osteoclast extracellular matrix, it is a sensitive measure of bone formation. Nonetheless, it has been linked to osteoporosis.As OC has a role in lipid and glucose metabolism (S. B. Oh, 2019) Osteocalcin), a tiny percentage of it is released into the circulation. Osteocalcin is a bone protein that aids bone growth and calcium homeostasis while protecting against obesity. Lipids and insulin resistance in osteoporotic women (Kanazawa, 2018, Paschou, 2017).

On the other hand, the results found an increase in the level of Osteopontin, so the results of the research agreed with the findings of (Nawar., 2015) and (. Chang,2010) who indicated in their study an increase in Osteopontin in serum The blood of patients with osteoporosis, so the reason for the rise may be due to the primary role played by OPN as it is the main regulator of many metabolic and inflammatory diseases, such as diabetes, cardiovascular disease, osteoporosis, and obesity. Therefore, other studies have demonstrated that OPN is a protein that plays a role as a protective factor for pancreatic islets, through the appearance of complex cytokines and hormonal interference between bone and liver cells. And adipose tissue, affecting bone remodeling, energy metabolism, and glucose homeostasis (De Fusco., *et al* 2017).

Another study discovered that OPN has a role in bone strength and remodeling, and OPN serum has been shown to be a biomarker for early detection of osteoporosis in postmenopausal women, with a positive relationship with osteoporosis severity. Fodor and colleagues (2013). As for the level of OS, it was found through the results that there was a rise in its levels in the blood serum of women with osteoporosis.

Estimation of the level of interleukin-6 in the blood serum

Table (1) shows the mean ± standard deviation of the interleukin-6 level, which was (20.532 ± 6.536) ng / ml in patients with osteoporosis, while the control group (healthy women) was (10.230 ± 1.987) ng / ml.

As shown in Figure, the results revealed a significant rise in the amount of interleukin-6 in the serum of osteoporosis patients compared to healthy women at the level of probability P ≤0.05. (5).

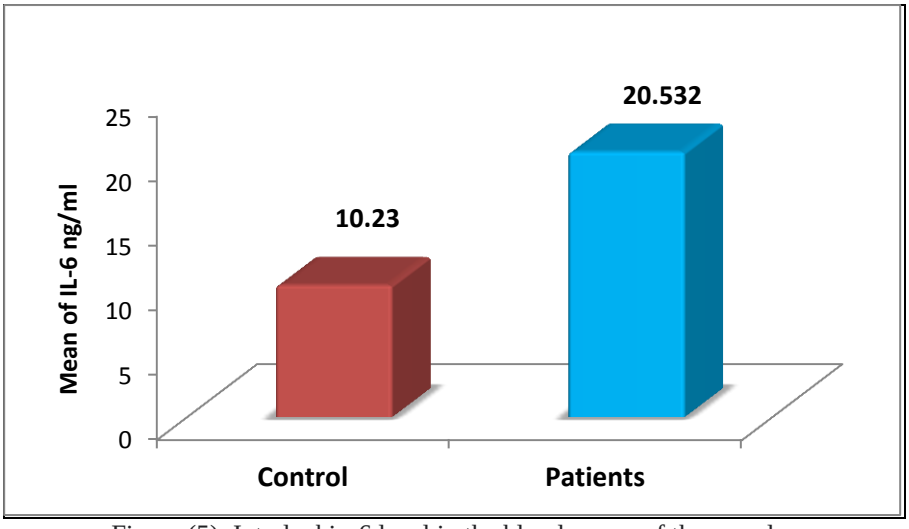

Figure (5): Interleukin-6 level in the blood serum of the samples

Estimation of the level of interleukin IL-1β in the blood serum

Table (1) shows the mean ± standard deviation of the level of interleukin IL-1β, which was (683.26 ± 236.06) ng / ml in patients with osteoporosis, while the control group (healthy women) was (208.69 ± 67.06) ng / ml.

The findings revealed a substantial rise in the amount of interleukin IL-1 in the serum of osteoporosis patients compared to healthy women at the level of probability P 0.05, as shown in Figure (6).

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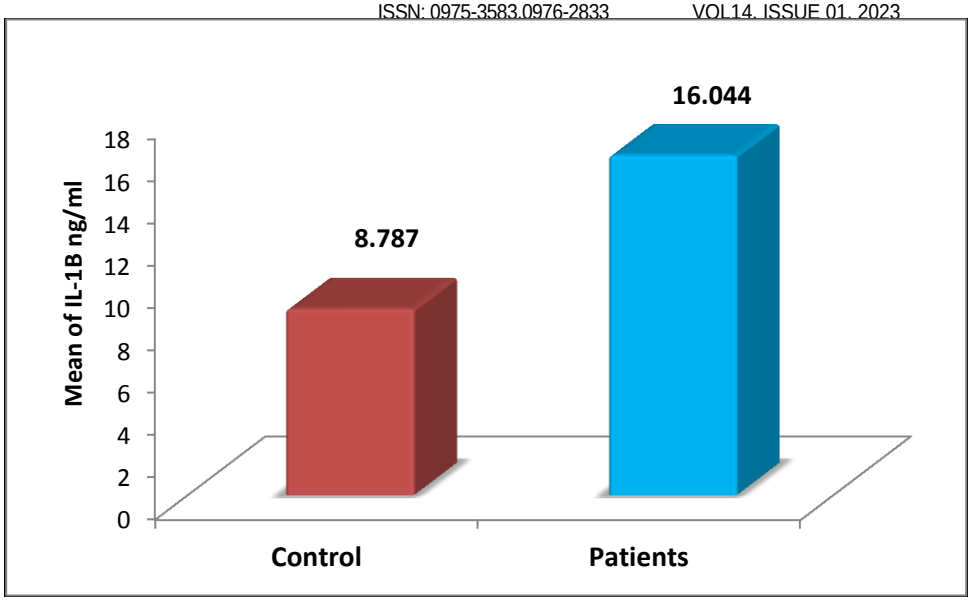

Figure (6): - Interleukin IL-1β level in serum samples

It was found through the results that the level of interleukin-6 increased significantly in the serum of patients with osteoporosis, so the results of the study agreed with the results of (Wiegertjes, *et al*. 2020) Significant Rise in the level of interleukin-6 in patients with osteoporosis.

Interleukin-6 is a versatile cellular cytokine that acts as a critical mediator in many acute inflammatory responses.Including activation of inflammatory lymphocytes and stimulation of hepatocytes to synthesize acute phase proteins. Blood except in acute infections, and the increase in it contributes too many diseases, especially in elderly people, such as osteoporosis, lymphoma and Alzheimer's (William.*et al* 2012).Therefore, interleukin-6 is one of the acute proteins, as its concentration increases in the blood serum during several hours after infection with bacteria and in turn provokes the synthesis of C-reactive protein. Therefore, interleukin-6 is an indicator of the presence of acute infections (Harbarth*et al*, 2001).

These findings indicate that inflammation has a role in bone metabolism and accelerated bone loss caused by estrogen deprivation during menopause (Murad *et al*., 2018).

Increased IL-6 production has been linked to osteoporosis in recent studies. Increased soluble IL-6 receptors have been described as a prognostic indication in assessing osteoporotic hip and bone fracture risk, and there is a substantial relationship between blood IL-6 levels, CRP, and BMD (Barbour. et al 2012).

On the other hand, it was determined through the results that there was a rise in the level of interleukin IL-1, therefore our findings agreed with those of (Eman et al., 2010) and (Tonia,*et al*., 2019). The researchers discovered aa notably high rise in the level of interleukin IL-1 in osteoporosis patients. Interleukin IL-1 is a type of cytokine that is important in immunology because it is primarily associated with innate immunity and relies on the functions of T cells and B cells, as innate immunity is manifested in infections, which can act as a host's defense mechanism but is detrimental to survival when out of control.Every member of theTLR family contains the cytoplasmicand IL-1 receptor Toll-IL-1-receptor. Interleukin 1β acts as a potent antibiotic antagonist, although its inflammatory properties can dominate innate immunity (Charles. 2017). Therefore, the elevated level of IL-1β may be due to the promotion of low-grade inflammation by estrogen deficiency and aging often, and the pro-inflammatory cytokines resulting from exacerbation of inflammation lead to osteoporosis and disease deterioration (Cline- Smith *et al*., 2020).

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