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Serum Levels of Sclerostin and Bone Turnover Markers in Children with Physical Disabilities

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ABSTRACT

Sclerostin is a hormone that inhibits bone formation via inhibiting Wnt signaling pathway. This study aimed to evaluate serum sclerostin levels in physically disabled children and to investigate its correlation with bone turnover markers (BTMs). This cross-sectional study included 68 Saudi physically disabled children (32 boys and 36 girls) and 79 apparently healthy children (39 boys and 40 girls), aged 6-12 years old. Serum levels of sclerostin, procollagen type I amino terminal peptide (PINP), carboxy-terminal crosslinked telopeptide of type I collagen (β-CTX), bone alkaline phosphatase (B-ALP), 25-hydroxy vitamin D (25(OH) D), and parathyroid hormone (PTH) were measured using ELISA kits. The median serum levels of sclerostin, PINP, B-ALP, calcium were significantly higher, while serum levels of β-CTX, 25(OH)D and PTH were significantly lower in patients versus healthy control. In boys, serum levels sclerostin, PINP, calcium and phosphate were significantly higher, while BMI, β-CTX, 25(OH)D and PTH were significantly lower in patients versus control. Meanwhile, in girls, BMI, serum levels of sclerostin, PINP, B-ALP and calcium were significantly higher, while β-CTX, 25(OH)D and PTH were significantly lower in patients versus control. The elevated sclerostin level in physically disabled children is not mainly affected by sclerostin dysregulation, however, it could be also affected by mechanical load. Further studies are needed to establish the potential role of sclerostin in bone formation in physically disabled children.

Key words: 25 hydroxy vit D, Carboxy terminal crosslinked telopeptide of type I collagen (β-CTX), Disabilities, Parathormone, Procollagen type I amino terminal peptide (PINP), Saudi children, Sclerostin

Introduction

Disability is one of the fundamental societal and economical medical issues in Saudi Arabia. Providing early medical/rehabilitative care, gives a better chance of reducing the complications of disability, thus improving quality of life (The World Health Organization, 2011). Disability is divided into three levels; the first is the damage in body functions or structures, the second is a restriction in reading or movement, and the third is the inability to perform daily activities. Some children can be disabled from birth while others acquire disabilities later on due to factors such as illness, injury, or poor nutrition (World Health Organization, 2012). Children and adolescents with severe physical disabilities are at risk of developing osteopenia and fracture later in life (Fehlings *et al*., 2011). Children's bone health is of importance, and peak bone mass usually reached by 20–25 years of age. Some studies indicate that high peak bone mass reduces the risk of osteoporotic fractures later in life (Heaney *et al*., 2000). Lifestyle factors, as physical activity and nutrition, as well as chronic disorders and medications, affect bone mineral accrual (Heaney *et al*., 2000; Söderpalm *et al*., 2007). Muscle weakness is associated with decreased bone development (Söderpalm *et al*., 2012), which in turn leads to an increased risk of fractures (Frost, 2004).

Sclerostin, encoded by the SOST gene located on chromosome 17q21 (Choi *et al*., 2008), is a potent inhibitor of bone formation (Balemans *et al*., 2001; Staehling-Hampton *et al*., 2002; Li *et al*., 2008). Subsequently to the maturation and the initiation of bone mineralization, osteocytes produce sclerostin at the end of their differentiation stages (Bonewald, 2011). Chondrocyte (Winkler *et al*., 2003) and cementocyte (Van Bezooijen *et al*., 2009) cells that are embedded in the mineralized matrices, also produce sclerostin. Several animal studies have shown sclerostin levels are inversely proportional to

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bone mass (Li *et al*., 2008; Lin *et al*., 2009) and that production of sclerostin by osteocytes is dramatically reduced by mechanical loading in rodents (Robling *et al*., 2006; Robling *et al*., 2008; Lin *et al*., 2009).

The paradigm for the Wnt signaling pathway states that Wnt combined to a co-receptor complex including Frizzled receptor and low-density lipoprotein receptor-related protein (LRP)-5 or LRP-6, both present on osteoblasts. This binding stabilizes cytoplasmic β-catenin and causes it to move to the nucleus. Translocation of β -catenin, leads to genes transcription that promotes osteoblast proliferation, differentiation, and function, ultimately resulting in new bone formation. Several antagonists can inhibit this signaling pathway. Molecules like secreted frizzled-related proteins, Wif (Wnt inhibitor factor), and Cerberus combine Wnt and functionally block the pathway. Dickkopfs (Dkk1) and sclerostin, inhibit Wnt pathway by preventing the Wnt- Frizzled- LRP5 complex formation either by enhancing LRP5/6 co-receptor (Dkk1) internalization or by competitive binding to LRP5 (sclerostin) (Mao *et al*., 2002; Li *et al*., 2005). These studies have established the role of Wnt signaling antagonists in the pathogenesis of disuse osteoporosis and provide a basis for the regulation of bone responses to unloading via enhanced or reduced Wnt signaling due to mechanical stimulation or unloading, respectively.

Bone formation is reduced, and bone resorption is increased in patients with physical disabilities, who lose mechanical load due to paralysis of their limbs as a result of immobilization. This eventually leads to reduced bone mass and size (Lin *et al*., 1996; Unay *et al*., 2003). Histomorphometric studies showed an increase in osteoclasts number and enlargement of resorption cavities in immobilized patients (Vico *et al*., 1987).

The aims of the present study were to evaluate serum sclerostin level in young Saudi children with physical disability and also to study the correlation between serum sclerostin level with body mass index (BMI) and bone turnover markers as procollagen type I amino terminal peptide (PINP), bone alkaline phosphatase (B-ALP) and carboxy terminal crosslinked telopeptide of type I collagen (β-CTX).

Patients and Methods

Participants:

This cross sectional study was conducted from March 2013 to September 2014. The study comprised of 68 Saudi patients (32 boys and 36 girls). Their age ranged from 6-12 years. All the girls and boys were in the prepubertal stage. The study subjects suffered from physical disability, hemiplegia and quadriplegia caused by cerebral palsy and/or muscular dystrophy. They were recruited from the Disabled Children's Association, in Jeddah, Saudi Arabia. In addition, 79 Saudi apparently healthy control volunteers (39 boys and 40 girls) age and sex matched with patients served as controls.

The assessments were performed at the center of excellence for osteoporosis research (CEOR) and King Fahad research center, King Abdulaziz University, Jeddah, Saudi Arabia. The ethical approval was issued by the CEOR's Human Ethics Research Committee according to Declaration of Helsinki. The parents of the children received written, and verbal information concerning the aim and processes of the study, and all gave their written informed consent before participation. Each participant was interviewed using a standardized questionnaire to gather information on socioeconomic status, medical history, lifestyle, level of physical activity (under physical therapy) in leisure time sun exposure, and the use of vitamins and medications. Exclusion criteria included: renal, disease, liver disease, thyroid disorders, diabetes mellitus and any medications that affects bone metabolism.

Anthropometric measurements:

Height and body weight measurements were recorded at the time of venous blood sample collection. The weight was measured by a Seca Digital Chair (Seca GmbH & Co., Deutschland). It is a special medical scale designed to measure the weight of the physically disabled patients. Height was measured when the participants are lying on the bed using a tape measure. BMI was calculated as weight (kg)/height (m^2) and recorded.

Biochemical measurements

Specimen collection:

Venous blood samples (8ml) were collected at random time (10 a.m. to 13 p.m.) using winged infusion set technique in a plain tube. Blood was centrifuged at 2500 g for 15 min by using CLAY ADAMS Dynac II Centrifuge (Becton, Dickinson and Company: Franklin Lakes, New Jersey: USA). Serum was separated and stored at -80ºC until analysis was performed.

Measurement of biochemical markers of bone and mineral metabolism:

Serum however measured using enzyme-linked immunosorbent assay (ELISA) according to the manufacturer procedure (Biomedica Medizinprodukte GmbH & Co KG, Austria). The biotinylated anti sclerostin antibody was used. The absorption was measured at 450 nm by microplate reader ELx 808, Bio Tek, USA. Sclerostin concentration was obtained from the standard curve which was drawn by plotting the absorbance versus standard concentrations in pmol/l. Bone turnover markers, PINP and β-CTX (Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim, Germany) were measured using electrochemiluminescence immunoassay (ECLIA) (Elecsys e411 autoanalyzer, Roche Diagnostics GmbH, Sandhofer Strasse116, D-68305 Mannheim, Germany).

The PTH was analyzed using Elecsys e411 autoanalyzer using the commercial kit (Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim, Germany). The 25(OH)D was analyzed by direct competitive chemiluminescence immunoassay using LIASON Fully Automatic autoanalyzer (DiaSorin Inc., Stillwater, MN, USA). The serum levels of B-ALP activity was measured using kit and reagents supplied by Ortho-Clinical Diagnostics, USA using Vitros 250 Chemistry System Autoanalyzer (Ortho-Clinical Diagnostics-Johnson & Johnson Co., USA). Serum calcium and phosphate were measured by routine clinical chemistry assays.

Statistical analysis:

The statistical analysis was performed using the Statistical Package for Social Science (SPSS program for Windows, version 20) (SPSS Inc., Chicago, IL, USA). Median and interquartile range (25^{th} – 75^{th} percentiles) were used for non- normally distributed variables. Mann Whitney *U* test was used to compare the difference between two groups due to abnormal distribution of the data. Person correlation was made between sclerostin and measured parameters. *P*-value <0.05 was recognized as statistically significant.

Results

Biochemical measured parameters*:*

The median serum levels of sclerostin, PINP, B-ALP and calcium were significantly higher in patients versus healthy control (*P* =0.0001, *P* =0.0001, *P* =0.009 and *P* =0.0001 respectively). While serum levels of β-CTX, 25(OH)D and PTH were significantly lower in patients versus control (*P* =0.0001 for all) (Table 1).

In boys, serum levels of sclerostin PINP, calcium and phosphorus were significantly higher (*P* =0.0001, *P* =0.0001, *P* =0.0001 and *P* =0.002 respectively); while BMI, β-CTX, 25(OH)D and PTH were significantly lower (*P* =0.016, *P* =0.0001, *P* =0.0001 and *P* =0.0001 respectively) in patients versus control. In girls, BMI, serum levels of sclerostin, PINP, B-ALP, and calcium were significantly higher (*P* =0.0001, *P* =0.0001, *P* =0.0001, *P* =0.0001, and *P* =0.024); while β-CTX, 25(OH)D and PTH were significantly lower (*P* =0.0001, *P* =0.0001 and *P* =0.0001, *P*=0017, *P*= 0.001 respectively) in patients versus control (Table 2).

Relationships between serum sclerostin; clinical and biochemical measures:

Sclerostin levels were independent of age in the patient's groups of both sexes. In patients group sclerostin is negatively, weakly associated to FT4 (r = -0.287, P= 0.010). Within the group of patients of both sexes, the correlations between serum sclerostin and BMI, PINP, β-CTX and B-ALP was insignificant and week (Table 3).

Table 1: Comparison between the physical and biochemical characteristics of patients and controls.

Variable	Control (n=79)	Patients (n=36)	*P –value using Mann Whitney U tests*
Age (years)	8.00 (8.00 - 9.50)	9.00 (8.00-10.00)	0.176
BMI (Kg/m²)	15.98 (14.94 - 16.86)	16.80 (14.78-19.18)	0.065
Sclerostin (pmol/L)	16.80 (14.27 - 20.16)	26.27 (14.63-40.19)	**0.0001**
PINP (ng/ml)	348.00 (296.40 – 418.90)	612.05 (482.58-799.18)	**0.0001**
β – CTX (pg/ml)	828.30 (755.00 – 948.00)	444.35 (298.50-609.50)	**0.0001**
B-ALP (U/L)	213.00 (199.00 – 226.00)	231.00 (203.00-266.00)	**0.009**
25(OH)D (nmol/L)	47.80 (40.20-56.60)	29.00 (17.65-36.68)	**0.0001**
PTH (pmol/l)	3.77 (3.14-4.22)	1.42 (0.97-2.16)	**0.0001**
TSH (µIU/mL)	1.91 (1.58-2.17)	2.96 (2.02-4.36)	**0.0001**
FT4 (pmol/L)	10.39 (9.66-12.08)	16.95 (14.95-19.25)	**0.0001**
Calcium (mmol/L)	2.41 (2.37-2.44)	2.59 (2.39-2.69)	**0.0001**
Phosphate (mmol/L)	1.44 (1.38-1.52)	1.56 (1.30-1.75)	0.125
Glucose (mmol/L)	4.92 (4.55-5.40)	6.90 (6.50-7.60)	**0.0001**
Creatinine (µmol/L)	29.00 (23.00-33.00)	44.20 (35.36-53.04)	**0.0001**
Albumin (g/L)	41.00 (40.00-43.00)	43.00 (41.00-45.00)	**0.0001**
ALT (U/L)	23.00 (20.00 – 26.00)	20.00 (17.00-45.00)	0.068

Data are represented as median (interquartile range). P values calculated using Mann Whitney U tests. BMI: body mass index; PINP: total procollagen type 1 amino-terminal propeptide; β-CTX: C-terminal cross-linking telopeptide of type I collagen. PTH: Parathyroid hormone, 25(OH)D: 25-hydroxyvitamin D, B-ALP: alkaline phosphatase.

Table 2: Comparison between the physical and biochemical characteristics of patients and controls boys and girls.

Variable	Boys			Girls		
	Control (n=39)	Patients (n=36)	P –value using Mann Whitney *U* tests	Control (n=40)	Patients (n=36)	P –value using Mann Whitney *U* tests
Age (years)	8.00 (7.00 - 9.50)	8.00 (7.00 - 9.00)	0.986	8.00 (8.00 – 10.00)	9.50 (8.00 - 11.00)	0.064
BMI (Kg/m²)	16.84 (16.15 – 18.05)	15.63 (13.98 – 17.19)	0.016	14.94 (14.70 – 15.53)	17.66 (16.04 – 20.12)	0.0001
Sclerostin (pmol/L)	20.10 (17.00 – 24.10)	22.27 (14.21– 42.32)	0.367	14.80 (12.67 – 16.70)	28.42 (15.04– 39.61)	0.0001
PINP (ng/ml)	328.00 (294.00 – 401.00)	548.90 (460.90 – 712.45)	0.0001	357.50 (298.85 – 484.25)	644.75 (486.50 – 827.93)	0.0001
β – CTX (pg/ml)	888.00 (811.00 – 949.00)	438.30 (248.55 – 604.30)	0.0001	822.65 (715.00 – 937.50)	518.60 (338.80 – 610.90)	0.0001
B-ALP (U/L)	213.00 (199.00 – 231.00)	231.50 (196.75 – 264.75)	0.252	211.50 (196.75 – 224.75)	231.00 (205.00 – 277.25)	0.017
25(OH)D (nmol/L)	49.10 (44.80-60.13)	29.00 (19.13-37.13)	0.0001	42.20 (34.35-51.26)	27.65 (17.15-36.68)	0.0001
PTH (pmol/l)	4.05 (3.66-4.58)	1.36 (0.70-2.16)	0.0001	3.15 (2.52-4.01)	1.72 (1.21-2.19)	0.0001
TSH (uIU/mL)	1.98 (1.79-2.16)	3.04 (2.01-4.58)	0.0001	1.77 (1.37-2.22)	2.86 (2.24-4.16)	0.0001
FT4 (pmol/L)	10.23 (9.02-11.11)	18.48 (16.50-20.20)	0.0001	11.44 (10.34-13.18)	15.90 (14.03-17.50)	0.0001
Calcium (mmol/L)	2.40 (2.36-2.41)	2.62 (2.39-2.70)	0.0001	2.41 (2.38-2.44)	2.52 (2.39-2.65)	0.001
Phosphate (mmol/L)	1.39 (1.30-1.45)	1.65 (1.39-1.92)	0.002	1.47 (1.43-1.52)	1.42 (1.25-1.69)	0.229
Glucose (mmol/L)	5.01 (4.66-5.34)	7.05 (6.80-7.80)	0.0001	4.83 (4.46-5.73)	6.60 (6.43-7.18)	0.0001
Creatinine (umol/L)	30.00 (27.00-33.00)	44.20 (35.36-53.04)	0.0001	28.00 (22.00-31.00)	44.20 (35.09-53.04)	0.0001
Albumin (g/L)	41.00 (40.00-43.00)	43.00 (41.00-45.00)	0.003	42.00 (40.00-43.00)	43.50 (41.00-45.00)	0.024
ALT (U/L)	25.00 (22.00 – 30.00)	20.00 (18.00 – 28.75)	0.050	21.00 (18.00 – 24.00)	20.00 (16.00 – 25.00)	0.620

Data are represented as median (interquartile range). P values calculated using Mann Whitney U tests. BMI: Body mass index; PINP: total procollagen type 1 amino-terminal propeptide; β-CTX: C-terminal cross-linking telopeptide of type I collagen, PTH: parathyroid hormone, 25(OH)D: 25-hydroxyvitamin D, B-ALP: alkaline phosphatase.

Table 3: Pearson's correlation between sclerostin level and variables in patients groups

Variables	Patients	
	R	*P*
Age (years)	-0.179	0.145
BMI (Kg/m²)	0.010	0.937
PINP (ng/ml)	-0.238	0.056
β – CTX (pg/ml)	-0.186	0.128
B-ALP (U/L)	-0.121	0.327
25(OH)D (nmol/L)	0.083	0.500
PTH (pmol/l)	-0.092	0.458
Calcium (mmol/L)	0.014	0.912
Phosphate (mmol/L)	-0.220	0.071

Data are expressed as correlation coefficient (r) and significance (P) was calculated using Pearson's correlation (weak correlation if $R < 0.3$, moderate correlation if $0.3 < R < 0.7$, strong correlation if $R > 0.7$). PINP: total procollagen type 1 amino-terminal propeptides. β – CTX: C-terminal cross-linking telopeptide of type I collagen, PTH: Parathyroid hormone, 25(OH)D: 25-hydroxyvitamin D, BMI: body mass index. B-ALP: alkaline phosphatase.

Discussion

The results of the present study showed that the serum sclerostin levels were higher in control boys compared to girls. Our results were similar to Kirmani *et al*. (2012) who conducted their study on healthy children (62 girls and 56 boys) aged 6-21 years old during growth. They stated that the serum sclerostin concentrations in boys were higher than girls, the change in its levels, according to gender, was shown during the period of adolescence, with a decrease in late puberty in both girls and boys. Kirmani *et al*. (2012) indicated that the levels of serum sclerostin were due to the cortical porosity, and hence the fluctuations in sclerostin synthesis throughout the growth period could be a part of defining the cortical structure.

The results obtained by this study revealed that serum sclerostin levels in young patients with physical disability, hemiplegia, and quadriplegia, were significantly higher than the control subjects. Meanwhile, serum levels of measured bone formation markers as PINP and B-ALP were significantly higher; while bone resorption marker as β-CTX was significantly lower in patients than healthy control regardless of the rise in sclerostin levels. In this respect, it was reported that B-ALP has a linear relationship with osteoblast and osteoblastic precursor activity (Reif *et al*., 2016). Bone alkaline phosphatase is a ubiquitous enzyme that plays an important role in the osteoid formation and mineralization (Delmas *et al*., 2000). It should be noted that these indices of bone activity are somewhat non-specific.

The intact PINP molecule is the amino end of type I procollagen before excision, and the formation of fibrils is a measure of the total synthesis of collagen in the body, all of which is related to the bone matrix (Delmas, 1993; Raisz *et al*., 1998). Several studies showed that sclerostin levels change in response to partial or complete mechanical unloading in human subjects (Amrein *et al*., 2012; Spatz *et al*., 2012). Spatz *et al*. (2012) showed increased in sclerostin levels, decrease in serum PTH, increase in urinary bone resorption marker and calcium, and decrease in bone mineral density (BMD) in healthy adults with prolonged skeletal disuse for 90 days. Morse *et al*. (2012) reported that circulating sclerostin levels were lower in patients with complete spinal cord injury who use wheelchair than in patients with an incomplete injury who did not use a wheelchair. Meanwhile, other researchers reported that patients with cerebral palsy show significantly lower circulating sclerostin levels than healthy controls (Gaudio *et al*., 2010; Shin *et al*., 2017). Morse *et al*. (2012), suggested that lower sclerostin levels in chronic patients with complete paralysis were due to a greater loss of osteocytes in an acute stage, compared to patients with incomplete paralysis.

Our results recommend that the increase in bone formation in patients is not mainly caused by sclerostin but other factors may have an essential role in determining bone formation. Frost (1987) stated that ‘‘Bone is a remarkable organ that can sense and respond to alterations in its loading environment, with disuse resulting in bone loss and overloading resulting in bone augmentation’’ (Frost, 1987). Another interpretation of our results is by demonstrating the effect of mechanical unloads on increasing the expression of sclerostin as it was pointed out in an experimental study performed by Frings-Meuthen and his colleague. In their study sclerostin levels were increased in young male human volunteers who were immobilized due to bed rest (Frings-Meuthen *et al*., 2013).

Moreover, another study reported that the serum sclerostin in patients with heritable metabolic bone disorders, X-linked hypophosphatemic rickets and osteogenesis imperfecta (aged 1.6-25 yrs, n=30) had higher sclerostin levels than healthy control subjects (aged 1.2-26 yrs, n = 22) and fairly high lumbar spine areal bone mineral density (Palomo *et al*., 2014). However, the sclerostin levels in the serum of the patients suffering from osteogenesis imperfecta were comparable to healthy control subjects even with low lumbar spine areal bone mineral density. They proposed that the bone mass irregularities in the stated diseases are not due to sclerostin dysregulation (Palomo *et al*., 2014).

In the present study, the physically disabled children were performing the physical activity while the blood sample was being collected. The children have physical therapy for three weeks in 3-4 month intervals. The mechanical loading that is generated from the physical therapy could explain the high level of PINP and B-ALP and the low level of β-CTX in the patients. This theory, however, could not be confirmed since we did not assess the PINP and β-CTX levels before and after physical therapy. It has been reported that the PINP was significantly reduced during the mechanical unload (Frings-Meuthen *et al*., 2013). Furthermore, Adami *et al*. (2008) studied the effect of physical activity on BTMs

and found that the levels of the PINP had increased without changes in β-CTX levels in postmenopausal women after a month of exercise. They concluded that PINP could be more sensitive to the mechanical load than β-CTX (Adami *et al*., 2008).

In animal models, there is a dose-dependent decrease in osteocyte sclerostin expression with mechanical loading, whereas unloading increases SOST expression (Robling *et al*., 2008; Moustafa *et al*., 2011). Lin *et al*. (2009) showed that a deletion of the SOST gene renders mice insensitive to disuse bone loss. Ferrari and his group (Bonnet *et al*., 2009) demonstrated that periostin, a matricellular protein, is a prerequisite for SOST inhibition through mechanical loading. Sclerostin inhibition is a promising approach to maintained bone mass. A monoclonal antibody (Scl-Ab) had good results in rats and monkeys (Li *et al*., 2009; Agholme *et al*., 2010; Ominsky *et al*., 2010; Tian *et al*., 2010; Tian *et al*., 2011).

The results of this study showed that 25 (OH) D was significantly decreased in patients versus control. Vitamin D is important hormone for calcium metabolism and accretion of bone mass during growth and development (Boot *et al*., 2011). Vitamin D is produced by the skin when exposed to sunlight and in some foods especially fish. Children with physical disability may be less exposed to sunlight than healthy children (Henderson *et al*., 2002), some of these children have feeding problems (Dahlseng *et al*., 2012) and insufficient intake of calcium and vitamin D has been reported (Hillesund *et al*., 2010), even in tube-fed children (Duncan *et al*., 1999). Consequently, their vitamin D status may be deficient or insufficient, and this may contribute to poor mineralization of the bone. The increase serum calcium level reported in this study could be due to a lower degree of calcium incorporation within the skeleton in physically disabled patients as result of decreased bone mineralization. The increase in the calcium level is responsible for low levels of PTH observed in our patients.

The woman with osteoporosis had a significantly lower level of serum sclerostin compared to women without osteoporosis. Serum sclerostin levels were found to correlate positively with ponderal index, body weight, and fat mass (Sheng *et al*., 2012). Fischer *et al*., (2012) presented contradictory result, showed no correlation between serum sclerostin and BMI in children. In our study, we showed that the sclerostin levels were independent of age in the patient's groups of both sexes. Fischer *et al*., (2012) reported no correlation between serum sclerostin levels and age as well as a formula for calculating serum sclerostin SD scores. Our results showed a weak negative correlation between serum sclerostin and BTMs (PINP and β-CTX) in patient groups (boys and girls). A lack of a significant correlation between sclerostin and BTMs values suggest an imbalance between resorption and formation that leads to an uncoupling of the two processes.

Contradictory results were achieved by Gaudio *et al*. (2010) reported that higher sclerostin levels were negatively correlated with bone formation markers but positively correlated with bone resorption markers in patients who were immobilized in beds or wheelchairs after stroke. The study included 40 Caucasian postmenopausal patients aged 61-103 yrs. It is important to mention that our patients were in the age range that precedes puberty where the bone does not yet reach the split point of bone age. Therefore, the children's bone mass, hormones, growth factors, 1,25-dihydroxy vitamin D levels, muscle mass, and bone formation are still developing (Saggese *et al*., 2002) and the PINP and β- β-CTX change with age during childhood. Power *et al*. (2010) showed that osteocyte sclerostin was inversely correlated with alkaline phosphatase in musculoskeletal diseases as osteoarthritis and femur neck fracture.

Our study has some limitations. First, the number of participants and the age range. We were not able to get a substantial number of participants for this research. The children were still at an age where we needed to obtain consent from the parents. They were not willing to let their children take part in this study due to their disabilities. Secondly, we could not perform dual energy X-ray scanning (DEXA) for the children due to their health situation, performing the DEXA would help us form the full picture about their bone mass density status. Thirdly, the extraction of blood was also difficult. Fourthly, the study patients are not divided according to the duration of physiotherapy they received, as a number of patents included in this study is small. Future studies are needed in the future that includes a large number of patients with motor disabilities in which the grade of motor disabilities are divided into five grades.

The strength of this project is that it is the first study conducted in Saudi Arabia stating the correlation between serum sclerostin, BMI, and BTMs in physically disabled children.

In conclusion, our results showed an increase in the sclerostin level in mechanical unload cases that could lead to bone loss. The mechanism of bone regulation in physically disabled children needs further clarification. Sclerostin inhibition continues to be a promising targeted therapy in different settings of bone loss. Therefore, clinicians need to consider both exercise and anti-sclerostin treatments as a synergistic strategy to enhance the anabolic effects on bone in patients with physical disability.

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Author Contribution:

HSS proofread correct, edit and add important notes to the final version of the manuscript; gave full supervision, technical support, conceptual advice and analysis of the data. WA performed experiments, collected, analyzed data, statistical analysis, drafting the manuscript, Tables and Figures. All authors read and approved the final manuscript.

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