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Association between Vitamin D, P1NP, and BMD as major indicators of bone health in local population of Pakistan

Dr Nasima Zafar
Department of Physiology, Hamdard College of Medicine & Dentistry, Hamdard University, Karachi, Pakistan

Dr Sana Mahmood*
Department of Physiology, Hamdard College of Medicine & Dentistry, Hamdard University, Karachi, Pakistan
*Corresponding author

Dr Nargis Anjum
Department of Physiology, Karachi Medical & Dental College (KMDC), Karachi, Pakistan

Dr Kauser Abbas Saldera
Department of Physiology, CIMS, Gambat, Pakistan

Dr Seema Zafar
Department of Obstetrician & Gynecology, Indus Hospital, Badin, Pakistan

Dr Shariq Ahmed Hafiz
Department of Ophthalmology, Abbasi Shaheed Hospital, Karachi, Pakistan

***Abstract*---**It is commonly known that the Pakistani population has inadequate vitamin D levels, but its effects on bone health and the level of N-terminal peptide of procollagen (PINP) have not yet determined. The aim of the research work was to check the vitamin D grade of exercisers and non-exercisers and examine how it related to P1NP concentration, a biochemical marker of bone production, and bone mineral density. A random sampling technique was used to select males and females who were staying in neighborhood. They were between the ages of 18 and 35 years. 92 people of Pakistani ancestry made up the total population. With 46 people, Group A was a non-exercising control group. Group B has 46 individuals in it. Both of these groups were gathered for the data analysis. BMD, procollagen type-1 amino-terminal pro-peptide, calciferol, and other biological indicator of bone remodeling were measured in serum. (P1NP). Using

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broadband ultrasound attenuation, the quality of the bones was evaluated. Before training, the total 25(OH)D serum were considerably less in non-exercising group than they were during or after training. (Mean: 0.4992, Standard Deviation: -1.6075; P 0001). This was connected to a noticeably increased serum Vit-D level in the exercise doing group (P= 0.001). In the B group (the exercise group), P1NP activity was also significantly higher, indicating increased osteoblast activity and bone turnover. For the Vit-D pre-training, no discernible differences were found between the two groups. However, compared to group A or the control group, the exercising/post-training group had significantly greater serum P1NP and 5-hydroxy Vitamin D concentrations. This was not linked to noticeably lower bone quality or increased markers of bone resorption in the control group.

***Keywords*---**Vitamin D, P1NP, BMD, bone health.

Introduction

Calciferol, sometimes known as vitamin D, is a vitamin which is not soluble in water and soluble in fats. The main usual basis of “Vit-D” is dermal production, with minor amounts obtained from the dietary sources like fish, milk, eggs, offal and other dairy products. Vitamin D can be obtained from food but more from cutaneal production, are physiologically inert and need to be converted by enzymes into active metabolites (Pazirandeh & Burns, 2014). These sources produce ergocalciferol and cholecalciferol, which are then transformed into 25-hydroxyvitamin in the liver. Circulating calciferol is a reproduction of dietetic and topical sources. Megalin and cubulin are involved in the active reabsorption of 25-(OH)D into renal tubular cells, where it is filtered at the glomerulus and transformed into the powerful hormone *1,25-dihidroxyvitamin D* (calcitriol) via 1-hydroxylase enzyme. It is almost universally accepted in the reported literature that Vit-D insufficiency affects obese and overweight people. A lack of “vitamin D” increases the menace of dying from cardiac sickness, cancer, infectious diseases, and trauma, all of which can have catastrophic repercussions (Valle Coca, 2017). The strongest measurement that determine the vitamin D level, an analyst of strength of bone , as well as the sovereign interpreter of threat of several many long-lasting diseases is considered as “25(OH)D” serum. Based on factors related to bone health, a Vit- D amount of 31.9 ng/mL (79 nmol/L) is suggested as ideal for normal health (Gariballa, Yasin, Abluwi, & Al Essa, 2022).

In recent years, widespread reports of <vitamin D> insufficiency have been made diagonally all age groups (Kimball, Fuleihan, & Vieth, 2008). Mass density of bone is among the key indicators of a bone's capacity to bear freight and is correlated with markers of bone production, whereas markers of bone resorption are correlated with skeletal size and bone volume (Brukner, 1998). For girls, this occurs amongst 11-15 years; for boys, it happens in the age range of 14 and 17 (Theintz et al., 1992). Increases in procollagen I amino pro-peptide (PINP) show that rises in BMD happen in conjunction with markers of the bone production (Ginty et al., 2004). Calciferol is crucial for bone remodeling as evidenced by the

expression of receptors for Vit-D on bone cells (Zarei, Morovat, Javaid, & Brown, 2016). The biomarkers for formation and resorption of bone, serum carboxy-terminal telopeptide (sCTX) type 1 and N-terminal propeptide (PINP) procollagen type 1, could be used in order to measure the amplitude of bone remodelling. The most accurate functional measure of the status of this vital vitamin is believed to be Vit-D blood sera concentrations (Cashman et al., 2017). Changes in the metabolism of bones have the biggest impact on serum PINP. The quantity is substantially elevated in newborns and teenagers as compared to grownups. PINP (s-PINP) serum is a helpful marker to determine the activity of disease in the Paget's disease of the bone, osteoblastic bone metastasis, and the monitoring of osteoporosis medication (Koivula, Risteli, & Risteli, 2012). Another study demonstrated that procollagen I amino-propeptide [(P1NP)], a bone turnover marker (BTM), is higher in cases of low vitamin D levels (Papanastasiou et al., 2020). BMD and increased fracture risk are connected. Low BMD is an indicator of osteoporosis risk. This is generally known that Vit-D strengthens bones by promoting intestinal absorption of Ca^{+2} and P. This hormone affects bone in two ways: it helps the osteoid matrix mature and become mineralized, and it help in mobilization of phosphorus and calcium from the skeleton when needed (Kimball et al., 2008). Obesity, the metabolic syndrome, diabetes, and cardiovascular disease have all been linked to less serum vitamin D concentrations (Valle Coca, 2017). There are numerous reasons why people lack vitamin D. Reduced consumption or absorption, less sun exposure, elevated catabolism in the heart, demoted endogenic production, and confrontation of terminal end of nerve conduction to vitamin D are a few of these (Bess Dawson-Hughes, Drezner, Rosen, & Mulder, 2021). A balance between bone resorption and bone production is necessary for optimal bone health since bone tissue is a unique structure that is constantly renovation. Chronic bone resorption causes the bone mineral density (BMD) to decline over time (Grønborg et al., 2019). A balance between bone resorption and bone production is necessary for optimal bone health. Chronic bone resorption causes the bone mineral density (BMD) to decline over time (Shetty, Kapoor, Bondu, Thomas, & Paul, 2016). Bone biomarkers in the blood can quantify bone turnover, making them a possible cost-effective technique to supplement BMD tests in circumstances when BMD is not a practical endpoint, such as shorter-term studies (Wheater, Elshahaly, Tuck, Datta, & van Laar, 2013). Therefore, low levels of serum 25(OH)D below 30 nmol/L and above 50 nmol/L are common throughout the world and are linked to weak bones and reduced muscle strength (Grønborg et al., 2019). Osteopenia and fractures are significantly increased by subclinical vitamin D insufficiency. Its effect on the catabolism and anabolism of bones and bone mineral density (BMD), however, is yet under the debate (Nair et al., 2020). Considering the reality that Pakistan is a country where vitamin D deficiency is extremely widespread, very few research have observed at the connection between vitamin D, BMD, and P1NP. This study looked at the relationship of vitamin D with P1NP and BMD in local Pakistani ethnicities as key markers of bone health.

Objectives

The current study assessed skeletal well-being and vitamin D level in healthy Pakistani people in light of the contentious findings about the link between BMD and vitamin D levels in various ethnicities. By comparing the association between

regrowing bone indicators and BMD in vitamin D adequate, inadequate, and scarce people, the goal was to ascertain whether vitamin D deficiency or insufficiency has an impact on bone turnover and BMD.

Methods

This research involves intervention. This epidemiological longitudinal investigation was carried out at the JPMC, BMSI-Karachi physiology department. The Institutional Ethics Committee for Clinical Studies permitted the study procedure. In Boot camp, the research study was conducted. For the RCT, a sample size of 92 participants was determined. Each of the two study groups, A and B, will have 46 individuals. The participants' demographic information, which contained their height, age, body mass density (BMD), weight, was also noted. The study's participants included both males and females between the ages of 18 and 35 who had BMD greater than -2.5 SD. The approach of non-probability sequential sampling was applied. Individuals were recruited for the study based on the inclusion and exclusion criteria. Gender, age range of 18 to 35 years, and population with sedentary lifestyle and BMD more than -2.5 SD. Pregnant and nursing women, people with musculoskeletal disorders, people with cancer, people with liver and kidney illness, and people with comorbid conditions including diabetes and hypertension are among the excluded groups. The participation in the study was also prohibited for the following individuals. Individuals with any medical condition that would make exercise dangerous, individuals with a history of surgery that affected bone turnover in the six months prior to the study, individuals exposed to chemotherapy or radiation therapy, individuals taking medications that affect bone metabolism, individuals following a specific diet, taking specific supplements, or engaging in a specific exercise regimen, and individuals who refused to participate in the study. Following Boot (exercise) camp's selection standards, a total of 92 people were recognised. All participants were counseled, given their agreement, and randomly assigned to the subsequent sets.
GROUP A-Control Group having BMD more than -2.5 SD.
GROUP B –Exercising Group having BMD more than -2.5 SD.

Bone mineral density

A trained operator measured each participant's BMD using a Lunar iDXA densitometer (GE, Healthcare) at lumbar spine, its sub-regions and at the hip. T-score was used to express the outcomes of quality control processes that were accomplished in accordance with the manufacturer's guidelines. The manufacturer-provided phantom was used in a weekly calibration method to determine the instrument variation on a regular basis. Based on T-score, BMD levels were classified as normal, osteopenic, and osteoporotic in accordance with World Health Organization (WHO) strategies.

Results

Comparison Of BMD Among Different Groups at Different Intervals

The mean BMD in control group -A was-1.9217±0.26492 (table 4.5.1). The mean Bone Mineral Density in intrvational group -B at pre traning was -1.9283±0.4507, at mid traning -1.9091 ± 0.3337 and in post traning -1.6075 ± 0.4992 with (p=0.000)(table 4.5.2). In post hoc test shows pre training with post training and mid training with post training has highly significant (p=0.000) (table 4.5.3). The mean difference between exercising and non exercising is highly significant with post training (p=0.000) (table 4.5.4). Bone Mineral Density increase in interventional group significantly at 6th month.

Bone mineral density

Table 3.1 T-Score in Non Exercising (control) Group

Non-Exercising (Control) Group) -A	N	46
	Mean	-1.9217
	± SD	0.26492

Table 3.2. Comparison of bone mineral density in exercising (interventional) group

Groups	Statistic	BMD			F-value	p-value
		Pre Training (Day-1)	Mid Training (Day - 90)	Post Training (Day-180)		
Exercising (Interventional) Group) - B	N	46	46	46	9.769	0.000*
	Mean	-1.9283	-1.9091	-1.6075		
	± SD	0.4507	0.3337	0.4992		

Table 3.3. Post Hoc Tests for multiple comparison of Bone Mineral Density in Exercising (Interventional) Group

"BMD"		P-value
"Pre-Training"	Between Training	0.848
	After Training	0.000*
"Mid Training"	Before Training	0.848
	Following Training	0.000*
"Post Training"	Prior to Training	0.000*
	Middle Training	0.000*

Table 3.4. Comparison between Exercising and Non-Exercising groups

		Pre-Training	Mid Training	Post Training
Group-A vs Group-B	t-value result	0.311	0.479	4.487
	p-value result	0.756	0.633	0.000*

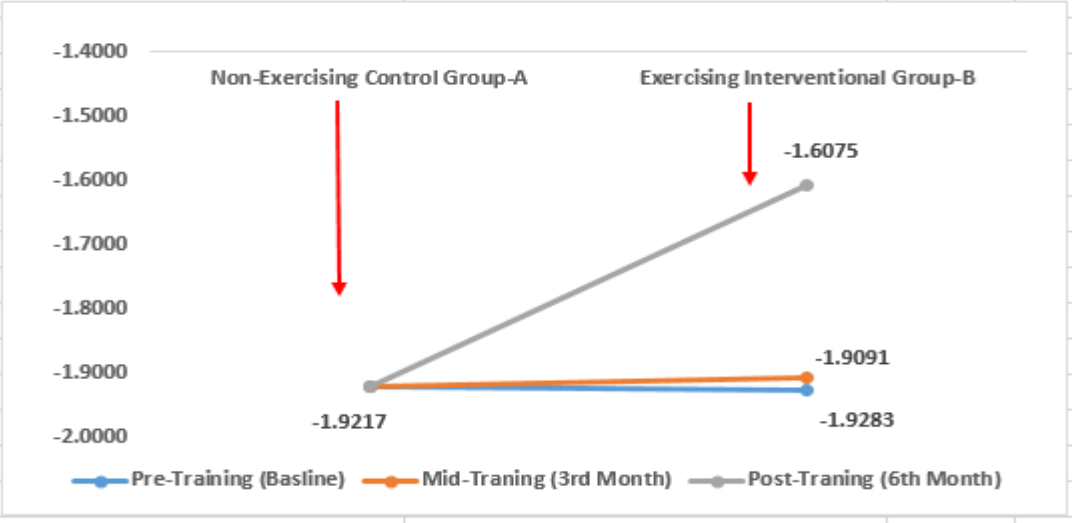

Figure 3.1 T-score Result for Baseline, (3rd. month) and 6th month in both Groups

The results showed the increased in bone mineral density in exercising interventional group B and there is no increase in BMD in non-exercising group A. These both peaks were compared with the pre-training values as baseline. Exercising had a great impact on increasing BMD.

P1NP Comparison between Various Groups at Various Intervals

The mean P1NP in control group -A was 41.442±17.792 (table 4.6.1). The mean P1NP in intrvational group -B at pre traning was 42.545 ±18.080 , at mid traning 58.626± 15.083 and in post traning 62.046 ± 15.181 with (p=0.000)(table 4.6.2). In post hoc test shows befor training with after training and before training with mid excercise has highly significant (p=0.000) (table 4.6.3). The mean difference between exercising and non exercisisg is highly significat with mid and post (p-0.000) and (p=0.007) respectively (table 4.6.4). P1NP increased in interventional group significantly at 3rd month.

PINP

Table 3.5. P1NP in (control) Group

Non-Exercising (Control) Group) -A	number	46
	Mean	41.442
	± SD	17.792

Table 3.6. Comparison of P1NP in exercising (interventional) group

Groups	Statistic	Procollagen Type 1 N-Terminal Propeptide (P1NP) (ng/dl)			F- value	p-value
		Pre Training (Day-1)	Mid Training (Day - 90)	Post Training (Day-180)		
Exercising (Interventional) Group) - B	N	46	46	46	20.478	0.000*
	Mean	42.545	58.626	62.046		
	± SD	18.080	15.083	15.181		

The group that exercises has elevated amount of procollagen type I N-terminal peptide. As the bone formation marker, it promotes both bone production and bone strength. P1NP levels are higher, which is a sign that bones are forming following training (day-180).

Table 3.7. Post Hoc Tests for multiple contrast of (P1NP) (ng/dl) in both groups

(P1NP) (ng/dl)		P-value
Pre Training	"Mid Training"	0.000*
	"Post Training"	0.000*
Mid Training	"Pre Training"	0.000*
	"Post Training"	0.253
Post Training	"Pre Training"	0.000*
	"Mid Training"	0.253

Table 3.8. Comparison between Exercising and Non Exercising groups

		Pre exercise	Mid Training time	afterTraining
Group-A vs Group-B	t-value	0.295	3.789	2.759
	p-value	0.769	0.000*	0.007*

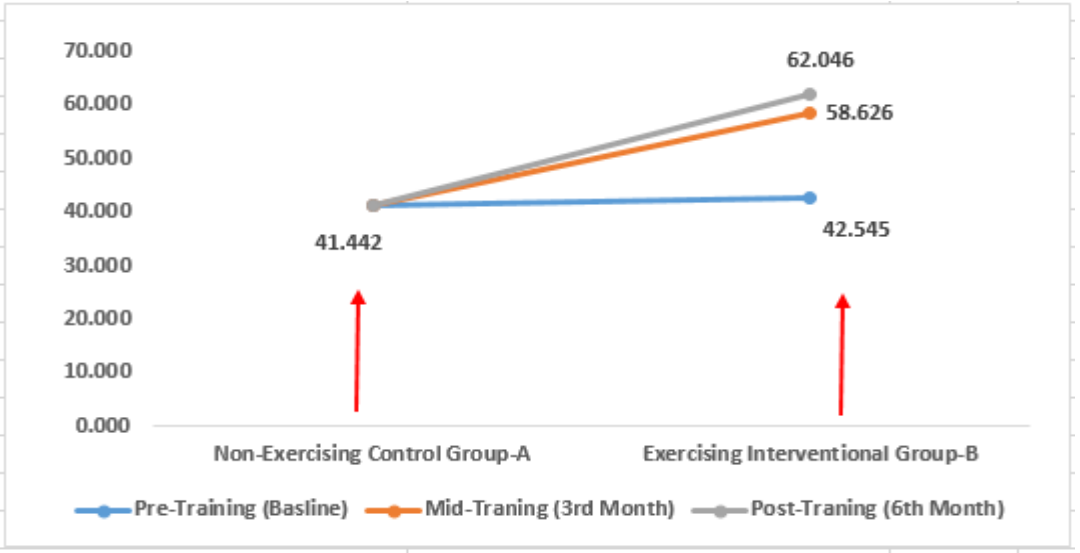

Figure 3.2. Evaluation of (P1NP) (ng/dl) in both groups under studied

P1NP is increased after 6 months of exercise. It is also seen that after 3rd month of training, this level started increasing. This graph is indicating that, at the baseline P1NP was 42 ng/dl. After Mid-training, its value was 58.6 ng/dl. But after post-training, P1NP was 62.04 ng/dl. These peaks also showing the positive impact of exercise on PANP level.

Vitamin D Levels Comparing Between Individuals

Before exercise, the levels of vitamin D were 14.70±2.5 ng/ml in the control group (A) and 14.34±2.4ng/ml in the interventional group (B), respectively, with a p value of (p = 0.491). Following exercise, vitamin D levels were still the same in the control group (A) and increased to 20.46±2.9 ng/ml in the intervention group (B), correspondingly, having p value results of (0.001). After exercise, vitamin D levels rose in the intervention group, with findings that were of statistical significance.

Table 3.9. Comparison of vitamin D (ng/dl) levels in the control (no exercise) and intervention (exercise) groups

Groups	Statisitic	Vit-D (ng/dl)		Mean of Difference (ng/dl)	t-value	p-value	95 % CI** of difference	
		Pre Training (Day-1)	Post Training (Day-90)				L..C.L	U.C.L
Non-Exercising (Control) Group) -A	N	46	46	- 0.030 (n=46)	1.721	0.092	-0.065	0.005
	Mean	15.704	15.734					
	± SD	2.526	2.481					
Exercising (Interventional) Group) - B	N	46	40	-5.175 (n=40)	14.836	0.001*	-5.880	-4.469
	Mean	15.347	20.465					
	± SD	2.480	2.916					
Group-A vs Group-B	t-value	0.682	8.130					
	p-value	0.491	0.001*					

* : Significant difference
**C.I: Confidence Interval

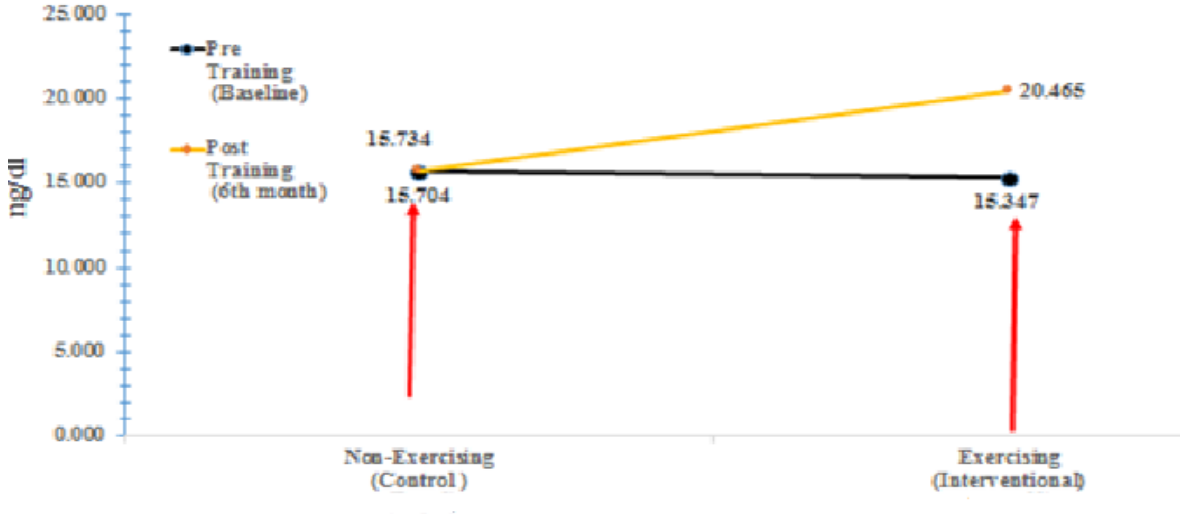

Figure 3.3. Comparing the levels of vitamin D (ng/dl) in the non-excersing versus the intervention groups that exercised

This graph is indicating that before training, Vit-D level was 15 ng/dl. Its value is same in both exercising and non-exercising group. Vit D level was enhanced after training (post training) from 15 to 20 ng/dl in exercising group. This indicated the positive impact of training on Vit D level.

Association of BDM (T-Score) with vitamin D
The corelation between BMD and vit- D was substantial. Or significant.

Table 3.10. Relation of BMD (T-Score) with Vitamin D

"BMD"	Variable	Interpretation or Correlation	P-value	Correlation or interpretation
BMD after training (T-Score)	post training (vit-D)	0.454	0.016*	• Very weak (0.00-0.199) • Weak (0.20-0.399) • Medium (0.40-0.599) • (0.61-0.798) Strong • (0.80-1.00)Very strong

*Significant value ($p < 0.05$)

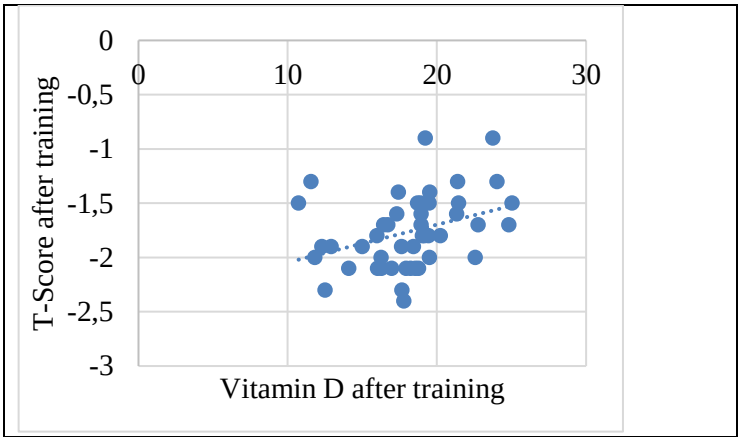

Figure 3.4. Relation of BMD with Vitamin D in accordance with t-score

This graph is indicating the positive relation between BMD and Vit-D. There is high validity and reliability of the data as the values are so close to the central point.

Association of P1NP with Vitamin D
P1NP and vitamin D were found to have a significant relationship.

Table 3.11. Relation of P1NP with the level of Vitamin (D)

P1NP value	Changeable factores(variables)	Correlation (r value)	Th P-value result	Relationship or correlation
<post training P1NP value (ng/ml)>	<Vitamin D post training>	<0.498>	<0.006*>	• Very weak vale of (0.01-0.19) • Weak (0.2-0.39) • Medium (0.4-0.59) • Strong (0.6-0.79) • Very strong (0.8-1.0)

*Significant value($p < 0.05$)

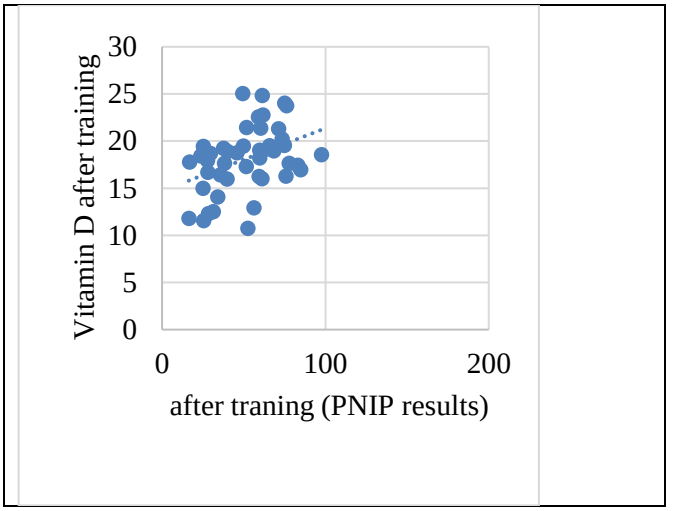

Figure 3.5. Relation of P1NP with Vitamin D

Vit D level was increased from 10 to 25 ng/dl after training. The P1NP was also seen to be increased in this case. So, these both markers had shown a positive correlation between them. They are directly proportional to each other. P1NP is mandatory for normal level of Vit-D.

Discussion and Conclusion

This study proved and indicated that (25(OH)D) is connected with bone mineral accretion, which may alter bone mass accumulation, as well as responsible for vitamin D deficiency that is present in both exercising and non-exercising groups commonly. These findings suggests that pre-training vitamin D levels have no impact on vitamin D status as determined by the serum 25(OH)D concentration after infancy. Randomized clinical trials have demonstrated that increasing vitamin D intake can enhance the group's members' gains in bone density. In addition to supporting bone health, it also has anti-proliferative impact on cells and regulates the immune system. The risk of bone disease, inflammatory illness,

and several cancers later in life is linked to Vit-D deficit individuals in early life; nevertheless, there are no long-term trials for intervention that support the widespread adoption of higher vitamin D consumption. In conclusion, inadequate vitamin D consumption need to be a global problem. Randomized trials are urgently required to establish the ideal status of vitamin D from foetal life via puberty. Moreover, maintaining and screening adequate status of vitamin D require public health awareness as well as a defined policy for clinicians and other healthcare professionals. Low 25(OH)D levels have no detrimental effects on bone health. Vit-D, BMD, and P1NP levels are positively correlated, and their values rise in the active group following workouts.

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