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RESEARCH ARTICLE

"USEFULNESS OF THERMO LABILE ALKALINE PHOSPHATASE AS A BIOMARKER IN DIAGNOSIS OF POST-MENOPAUSAL OSTEOPOROSIS"

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Key words:-
Osteoporosis, Menopause, Biomarker

Abstract

Background: Osteoporosis is characterized by demineralization of bones and it is a complication of women in post-menopausal life. Bone alkaline phosphatase is an isoenzyme of alkaline phosphatase which helps in the mineralization of bones and it is heat labile in nature. Its measurement facilitates the clinical diagnosis of Osteoporosis.
Objectives: To assess the status of total alkaline phosphatase and heat-labile isoenzyme fraction (bone fraction) in serum of pre and post-menopausal women and to relate the findings to the clinical condition /final diagnosis to make out the correlation between the pre and postmenopausal women.
Methodology:A total of 70 subjects, consisting of 35 pre-menopausal women (38.11 Â± 4.3 years of age) and 35 post-menopausal women (54.40 Â± 4.6 years) were constituted for the study. Their total alkaline phosphatase was measured directly and the heat stable alkaline phosphatase was measured after heating the serum at 56℃ for 10 minutes. Spectrophotometric P- Nitro-Phenyl Phosphate method was followed for the estimation. Heat labile alkaline phosphatase levels were obtained by subtracting the heat stable fraction from total Alkaline phosphatase.
Result: The measured serum total alkaline phosphatase level was slightly increased in post menopause compared to premenopausal women but it was not statistically significant. The levels of heat-labile Alkaline phosphatase showed significant difference between pre and post-menopausal women by ***: $p < 0.01$: ***: $p < 0.001$ respectively.
Conclusion: Heat labile alkaline phosphatase can be used as a biomarker to diagnose Osteoporosis in post-menopausal women.

Introduction:-

Osteoporosis is a systemic skeletal disorder characterized by a reduction in bone mass and demineralization of bone tissue, leading to weak bones and increased susceptibility to fracture[1]. In India, the prevalence of this disease in post-menopausal female population in various studies varies between 25% and 62%[2]. The incidence of osteoporosis increases with age and there is a sharp increase in cases following menopause in females.[3]

The pathophysiology of Osteoporosis is complicated. Peak bone mass is attained at around thirty years of age. After the attainment of middle age, the resorption begins to exceed the osteoblastic activity. Normal loss of bone averages 0.7% per year. However at the time of menopause it increases to 2 – 5% per year, which may continue further up to

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ten years which leads to osteoporosis[4]. The diagnosis and monitoring of treatment for osteoporosis are routinely done by clinical assessment, radiography and bone densitometry. However, in recent years biomarkers of bone formation and resorption have been identified to quantify bone turnover and reconstruction, with possible applications in clinical practice[5,6]

Total alkaline phosphatase(TALP) activity in serum has been used commonly as a biomarker of osteoblasts function, but it lacks specificity because of the concentration of activity derived not only from bones but also from the liver[7].In a healthy population about half the activity of total alkaline phosphatase in serum is derived from bone and the remaining from the liver. The iso-enzyme forms differ only in the degree of sialylation and glycosylation, reflected in differences in electrophoretic mobility, heat stability and precipitation by lectin [7]. Bone alkaline phosphatase (BALP) is more heat labile than liver alkaline phosphatase and it can be inactivated by incubating the serum at 56^0C for 10 minutes [8]. This technique is simple, cost-effective and uses no chemicals to separate and quantify the particular isoenzyme fraction. There is an urgent need to study the bone fraction of ALP in post-menopausal women of the Bangalore Sub-population to know the homeostasis of calcium and phosphorus. Thus in the present study, we have attempted to estimate the serum ALP fractions in both pre and postmenopausal women.

Materials and Methods:-

A cross-sectional study was done in pre-menopausal and post-menopausal women who were attending at Padmashree Diagnostics, Department of Gynecology, Vijayanagar, Bengaluru for elective diagnosis and treatment. The study was approved by Padmashree Institutional review board. After obtaining the informed consent from each study participant, a total of 70 subjects including 35 each of pre-menopausal and post-menopausal women in the age groups of 38.11 ± 4.3 yrs and 54.40 ± 4.6 yrs respectively were constituted in the study. Sample collections, processing and data analysis were done as per the standard procedures.

Sample collection procedure:

The blood specimens received in the laboratory were centrifuged (1800 x g/15mins) to separate the cellular components and the cell-free serum was processed for the analysis of routine biochemical parameters sought by the treating clinicians. The remaining specimens were liquated, labeled and stored at – 20ºC till further analysis. Aliquots of specimens, once thawed were used for the analysis on the same day and not subjected to repeated freezing and thawing to avoid any pre-analytical errors.

Inclusion and Exclusion criteria:

Selection Criteria	Pre-Menopausal	Post-Menopausal
Inclusion Criteria :		
Age in Years	25 – 45	48 – 67
Gender	Female	Female
Healthy Volunteers	Yes	No
The menopausal woman who had cessation of menses for at least one-year duration	-	Yes
The woman who had menses at a regular duration	Yes	-
Not on Hormone Replacement Therapy	Yes	Yes
Exclusion Criteria:		
Metabolic diseases like hypo/ hyperparathyroidism, Hyperthyroidism, and diabetes mellitus.	No	No
Autoimmune diseases like SLE and rheumatoid arthritis	No	No
Surgery like gastrointestinal resection or malabsorption	No	No
Chronic liver or Renal diseases	No	No
Drugs that may affect bone metabolism like Glucocorticoids, aluminum-containing antacids, Frusemide, bisphosphonate, calcium, Vitamin A, Vitamin D, Calcitonin, lithium, antiepileptics and anticoagulant, hypercalcemia of malignancy	No	No

Chronic granulomatous diseases like sarcoidosis and tuberculosis	No	No
Paget's disease of bone	No	No
Cigarette smoking, Alcohol abuse	No	No
History of recent fractures (in the earlier six months)	No	No

Type of study:
Randomized control study

Research design:
Non-Interventional

Analytical Method:
Serum total ALP and heat stable fractions were estimated by the Spectrophotometric P- Nitro-Phenyl Phosphate method described by IFCC (International federation of clinical chemistry). For the estimation of heat stable ALP, serum was incubated at 56^0C for 10 minutes before assay. Heat labile ALP activity was obtained by subtracting the heat stable fraction from the value of serum total ALP.

Device Description:
COBAS Integra 400 plus – Random assess fully automated system was used for the estimation of ALP levels.

Procedural steps:
1. Fix the primary tube container into the rack, ensuring that the barcode labels were clearly visible through the slot in the rack.
2. Place the rack in the sample compartment.
3. Press the Start button.
4. The instrument was fully automated and analyzes the sample automatically.
5. The values were noted.
6. The samples were heated at 56^0C for 10 min in the water bath. Thawed to room temperature and loaded into the instrument and Heat stable fraction was measured.

Statistical analysis:
SPSS Version 19 Data analysis package was used and applied to analyze the obtained data after discussion with the Biostatistician. All the values were expressed in mean ± SD. Statistical comparison was performed using the student "*t"* test. The student's 't' test *$p< 0.05$; **$p< 0.01$; ***$p< 0.001$ was considered as significant.

Results:-

The measured serum total ALP level was slightly increased in post-menopausal women than in premenopausal women by 11% (69 and 62U/L Respectively). But it did not show any statistical significance (**Fig 1**). After subtracting the Heat stable-ALP (HS-ALP) fraction from Total ALP, the obtained Heat labile-ALP (HL-ALP) fraction, depicts (Figure 2) the statistically significant difference between pre and post-menopausal women by **: $p < 0.01$: ***: $p < 0.001$ respectively. Further, though the observed HL-ALP fraction in pre and post-menopausal levels was significantly lower than the referred reference interval but the post-menopausal fraction of HL-ALP activity was 20.5% more than pre-menopausal women. It indicates more bone remodeling or a higher rate of bone turnover/ bone loss.

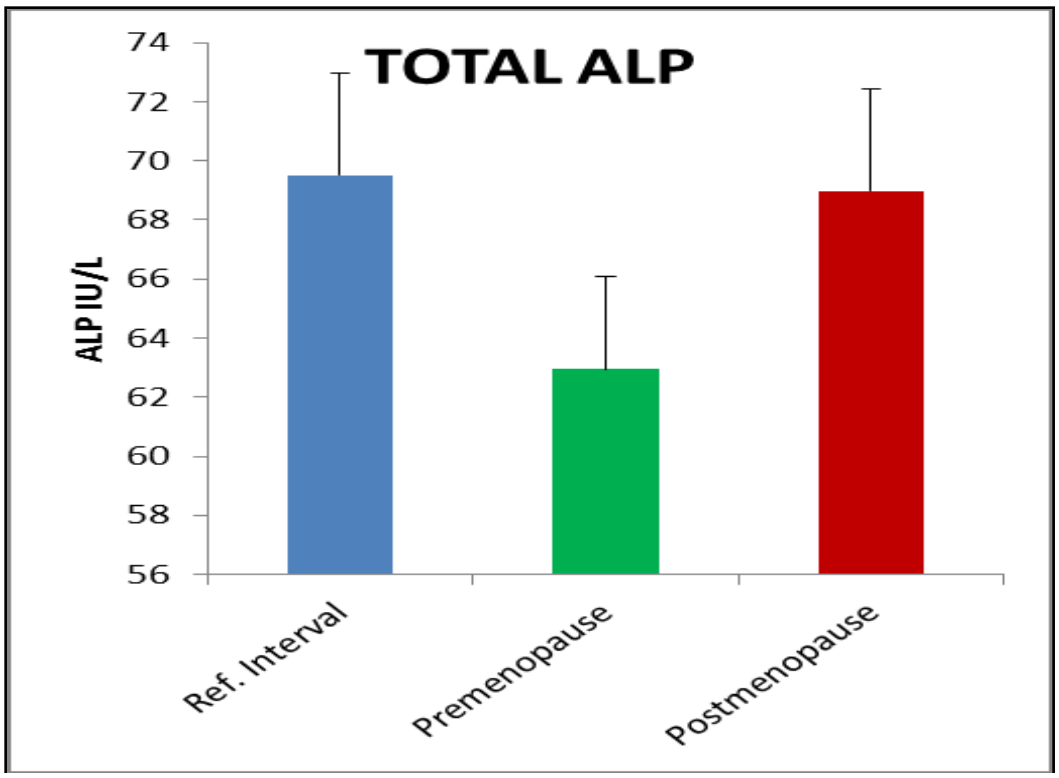

Fig 1:- Histogram represents the Serum Total ALP among the pre and post menopause women.Values expressed as Mean ± SD. Student's't' test: *: $p < 0.05$; **: $p < 0.01$: ***: $p < 0.001$.

Total ALP lacks the diagnostic specificity because of the total measurement of both the fractions like Bone as well as liver fractions. Heat labile ALP is much more specific than total ALP assay, because Heat labile ALP is bone-specific.

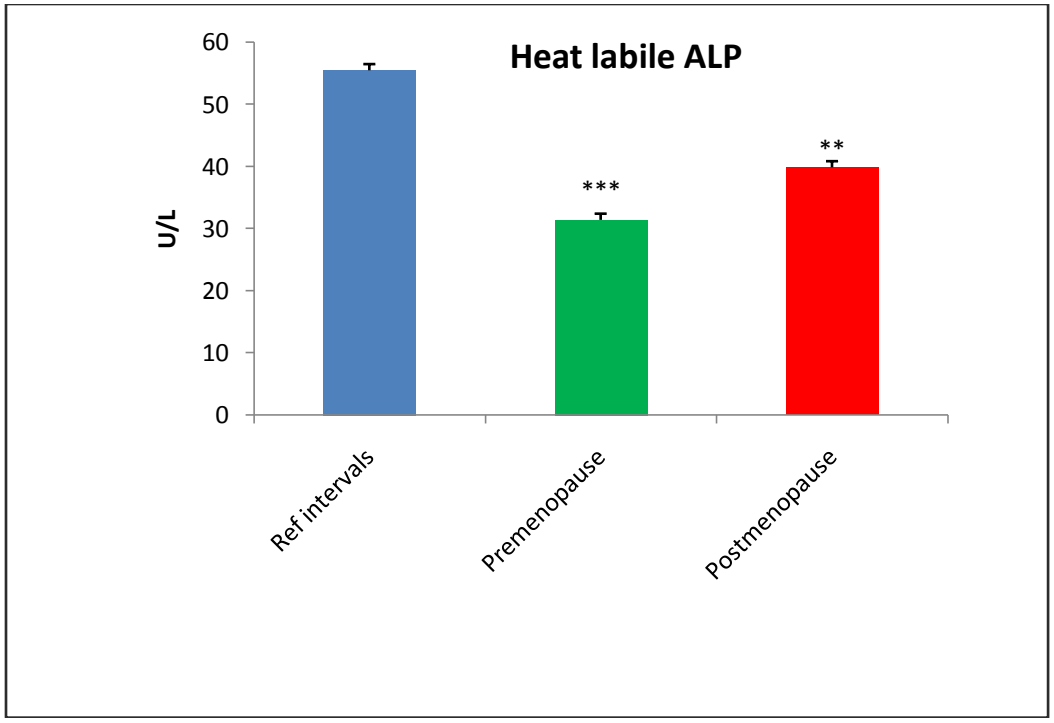

Fig 2:- Histogram represents the Serum HL-ALP among the pre and post menopause women.Values expressed as Mean ± SD. Student's't' test: *: $p < 0.05$; **: $p < 0.01$: ***: $p < 0.001$.

In the current study available reference intervals were subjected to assess the level of heat-labile fraction activity in pre and post-menopausal women. Hence, the study correlated the pre and post-menopausal women's HL-ALP fraction activity did reveal its statistical significance $^{**}p < 0.01$in post-menopausal women population by a 20.5% increase(**Fig 1 &2**). This underscores the high degree of bone turnover or remodeling activity in post-menopausal women.

Discussion:-

Total ALP is one of the most often sought out investigation as a nonspecific marker of osteoporosis. Fractional ALP i.e., HL- ALP is much more specific than total ALP assay, because HL- ALP is bone-specific. For those women who are in post-menopause, decreased estrogen level leads to decreased Vitamin D and decreased absorption of calcium. Because of the negative feedback mechanism, the parathyroid gland releases more parathormone which promotes the activity of osteoclasts resulting in more ALP secretion. It is diagnosed with an accurately measured total ALP and HL ALP fraction.

As the WHO data states that due to stupendous increase in life expectancy in India, it can increase the post-menopausal woman population to 173 million by 2026. Therefore there is an urgent need to focus our health care service on the post-menopausal women. The present study involves the main objective to evaluate serum HL-ALP fraction in pre and post-menopausal women and to evolve a reference range for the Indian sub-population. This may be a simplistic tool in primary health care testing laboratories which may not be equipped with to carry out study on high-end biomarkers.

The present study is an attempt to find a simple method which can open the scope for implementing it as an extended parameter HL-ALP fraction for the post-menopausal women population suspected to be suffering from osteoporosis. Since, plasma calcium levels are usually normal in osteoporosis, even if the bones are deficient, total and bone alkaline phosphatase can be used to monitor the rate of bone mineralization. Increased HL-ALP fraction in a post-menopausal women is a major risk of accelerated bone loss. Women tend to lose about 1% of bone density annually during and after menopause. Nearly 35% of women undergo bone loss at a faster rate during the perimenopausal phase. These biochemical parameters may provide an overview of the rates of osteoblast and osteoclast activity.

Conclusion:-

In the present study, the total ALP did not show any significance in the post-menopausal women population when compared to the premenopausal group. By and large, the observed HL-ALP fraction was found to be significantly ($^{**}p<0.01$) increased in post-menopausal women. These findings suggest that the prerequisite pharmacotherapy for bone remodeling or bone turnover could resolve the clinician's diagnostic dilemma by enhancing the accuracy and credibility of drug prescription.

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Conflicts of Intrest

The authors of the study have no financial interest nor received any financial support from the companies that manufacture reagents and instruments for the estimation of ALP.

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