Journal Homepage: - www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI: 10.21474/IJAR01/12566
DOI URL: http://dx.doi.org/10.21474/IJAR01/12566

RESEARCH ARTICLE

"A STUDY ON STATUS OF URINARY HYDROXYPROLINE IN POST MENOPAUSAL WOMEN"

Lalrintlingi Sailo, Apurba Sarkar and Dr. Suresh Babu

Manuscript Info

Manuscript History
Received: 10 January 2021
Final Accepted: 14 February 2021
Published: March 2021

Abstract

Introduction: WHO and the Stages of Reproductive Aging Workshop have defined menopausal transition as the time of an increase in follicle-stimulating hormone and either increased variability in menstrual cycle length, two skipped menstrual cycles with 60 days or more of amenorrhea, or both. It concludes with the final menstrual period. Post-menopause begins at that time, although it is not recognized until after 12 months of amenorrhea. During menopause, women face various physiological, psychological, and biochemical changes. Laboratory medicine has given a new background to overcome the clinicianÃ‚Â's diagnostic dilemma. Hydroxyproline is mainly found in collagen and accounts for 13% of total amino acid content and derived from proline by post-translational hydroxylation. Hydroxyproline is derived from another amino acid such as proline. Direct urinary assay of hydroxyproline to measure bone resorption have clinical applications as part of screening programs to assess the risk of osteoporotic fractures. Method: A total of seventy patients with regular medical follow-up records, The Patients were pre and post-menopausal women (35 each) recruited for this study. Patient details like body mass index, education, smoking, alcohol intake, dietary habits, and family history were considered before selecting the patients. Analytical Methods: Urinary Hydroxyproline and Urinary creatinine was estimated by Modified Neumann et al and Spectrophotometric JaffeÃ‚Â's reaction respectively. Result: The study population consisted of 70 participants of premenopausal (n=35) and postmenopausal women (n=35), mean age of 38.11 Ã‚Â± 4.3 and 54.40 Ã‚Â± 4.6 respectively. The bone mineralization marker urinary total hydroxyproline was quantified in pre and post-menopausal women, which is 80.3 Ã‚Â± 75mg/L and 136 Ã‚Â± 103mg/L respectively. The urinary creatinine level in pre and post-menopausal women was 53.7 Ã‚Â± 14.2mg/dL and 37.0 Ã‚Â± 27.3 respectively. The hydroxyproline: creatinine ratio (HCR) was 41% to 69% against the normal reference interval in pre and post-menopausal women. Conclusion: The obtained normative data for the premenopausal woman population would be a new reference range in Indian sub-population or otherwise general population normative reference range commonly being used as a reference interval in all kind of pathophysiological disorders. Hence, the derived parameter confirmed

Corresponding Author:- Apurba Sarkar

that HCR is the most prognostic significant diagnostic marker in pre and post-menopausal patients.

……

Introduction:-

The World Health Organization (WHO) and the Stages of Reproductive Aging Workshop have defined menopausal transition as the time of an increase in follicle-stimulating hormone and either increased variability in menstrual cycle length, two skipped menstrual cycles with 60 days or more of amenorrhea, or both. It concludes with the final menstrual period. Post-menopause begins at that time, although it is not recognized until after 12 months of amenorrhea [1]. During menopause, women face various physiological, psychological and biochemical changes.

The adverse effects of menopause are attributed to a decrease in the estrogen level, which may lead to alternations in body mass index, insulin levels and also to increase the risk of hypertension, cardiovascular diseases, osteoporosis, diabetes mellitus, cancer and other degenerative changes in postmenopausal females [2]. Osteoporosis one of the major debilitating and hilarious issue in recent medical treatment due to various advancement in imaging technology approaches like bone densitometry, a biopsy of bone and biochemical assays provide an insight understand the pathophysiological changes in bone remodeling and disorders like osteoporosis [3]. However, today the laboratory medicine has given new milieu to overcome the clinician's diagnostic dilemma. Like the biomarkers, Calcium, phosphorus, ALP, osteocalcin, Vitamin D, Pyrilinks, PTH, N-telopeptides, C-telopeptides and urinary total and free hydroxyproline and proline parameters reveal changes in bone metabolism much earlier, than the radiographic methods.

Hydroxyproline is mainly found in collagen and accounts for 13% of total amino acid content and derived from proline by post translational hydroxylation. Hydroxyproline is derived from another amino acid such as proline [4]. It is the major turnover product of collagen, the main protein of the bone matrix [5]. It is created by the interaction of ascorbic acid, also known as vitamin C, and proline. This causes a hydroxyl group, which is a bonded oxygen-hydrogen molecule, to attach itself to the carbon atom of the proline acid, changing it into Hydroxyproline [6]. Defects in collagen synthesis lead to easy bruising, internal bleeding, breakdown of connective tissue of the ligaments and tendons, and increased risk to blood vessel damage. Increased spill of hydroxyproline in the urine is generally associated with breakdown of connective tissue due to disease process and may also be a manifestation of vitamin C deficiency [7]. The major hallmark in postmenopausal woman is decrease in Estrogensynthesis, where in increases the rate of bone remodeling, which results in high turnover bone loss [8]. There are recognized receptors on the osteoblast which do not function optimally due to lack of estrogen. This is reflected by a significant increase in the mean value of markers of resorption from pre-menopause to post-menopause [9], [10]. Thus simple, direct urinary assay of hydroxyproline to measure bone resorption have clinical applications as part of screening programs to assess the risk of osteoporotic fractures [3], [11].

Objective Of The Study:-

1. To assess the status of urinary Hydroxyproline in Postmenopausal women.
2. To correlate these biochemicals as a marker of osteoporosis in post menopausal women.

Materials and Methods:-

Materials:

Patients who were attending at Padmashree Diagnostics, Department of Gynaecology, Vijayanagar, Main Road, Bangalore, for elective diagnosis and treatment, who were belonging to pre and postmenopausal woman recruited for this study. After complete explanation of the study to the subjects, a written informed consent was obtained from subjects. A total of **seventy** pre and postmenopausal women (35 each) was recruited for the present study. The study proposal was approved by Institutional Review Board, Padmashree Institute of Clinical Research. Bangalore.

Following criteria were used to include or exclude subjects for the study:

Selection Criteria	Pre Menopausal	Post Menopausal
Inclusion Criteria :		
Age in Years	25 – 45	48 – 67
Gender	Female	Female

Healthy Volunteers	Yes	No
Menopausal woman who had cessation of menses for at least one year duration	-	Yes
Woman who had menses at regular duration	Yes	-
Not on Hormone Replacement Therapy	Yes	Yes
Exclusion Criteria:		
Metabolic diseases like hypo/ hyperparathyroidism, Hyperthyroidism and diabetes mellitus.	No	No
Auto immune diseases like SLE and rheumatoid arthritis	No	No
Surgery like gastrointestinal resection or malabsorption	No	No
Chronic liver or Renal diseases	No	No
Drugs which may affect bone metabolism like, Glucocorticoids, aluminium containing antacids, Frusemide, bisphosphonate, calcium, Vitamin A, Vitamin D, Calcitonin, lithium, antiepileptics and anticoagulant, hypercalcaemia of malignancy	No	No
Chronic granulomatous disease 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

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

Methods:
Patient Selection:
A total of **seventy** patients with regular medical follow up record, The Patients were pre and post menopausal woman (35 each) recruited for this study. Patient details like body mass index, education, smoking, alcohol intake, dietary habits and family history was considered before selecting the patients.

Sample Size:
Two Groups of 35 Subjects each

Sampling method:
Random Sampling

Analytical Methods:
Urinary Hydroxyproline and Urinary creatinine was estimated by Modified Neumann et al [12] and Spectrophotometric Jaffe's reaction respectively.

Sources Of Data:
Available literature information from recent publications was updated during the course of study. The study design to be employed was standardized /modified depending upon the situation before applying the same for the sample analysis. Information with respect to study outcome was procured from the patient medical records and clinical expertise opinion was sought before relating the study outcome.

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 student t test. The student's 't' test *$p< 0.05$; **$p< 0.01$; ***$p< 0.001$ was considered as significant.

Assay Protocols :
The analytes of interest in urine were assayed by the following methods. In general, the methods followed are described in detail with respect to the principle of the assay methods, nature of reagents, reference materials (Calibrators) and their source, instruments used and limitation of the assay methods if any with appropriate references, in the following sections. The urinary Hydroxyproline assay method based on Neumann et al., [12] was standardized and validated for, and confirmed to have the required detection range of 15 - 250 mg/L as depicted in (Fig 4.1).

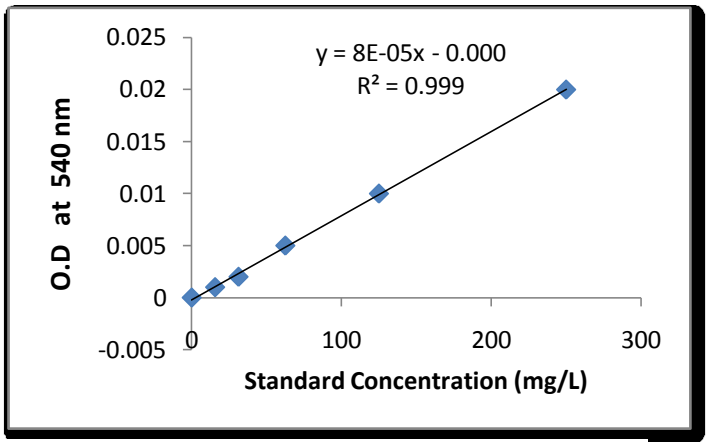

Fig 4.1:-Depicts the detection range (15 - 250 mg/ L)for Urinary Hydroxyproline by Neumann Method.

Analytical Methods:
Urinary Hydroxyproline – Total:
Principle:
The assay method is involves that urine sample(s) is/are subjected to hydrolysis to convert the Hydroxyproline content of peptides/proteins to free amino acids. The present method has brought some modification, instead of conventional procedure of acid hydrolysis at 100^0C for overnight period, current method involve the hydrolysis of Hydroxyproline content of peptides/proteins by autoclaving at 15 psi for 60 mins in alkaline conditions, After hydrolysis, the hydrolysate were adjusted to near neutral pH (~ 7.0 ± 0.1), and processed for assay of Hydroxyproline. In alkaline medium the urinary protein hydrolysate containing hydroxyproline in the presence of $CuSO_4$ and H_2O_2, results in the formation of pyrroline-4-carboxylic acid, which upon acidification is converted to pyrrole-2-carboxylic acid. The latter condenses with p-dimethylaminobenzaldehyde to give a colored complex which is measured at 540nm.

Chemicals / Reagents:
In-House prepared.

Reagents:

1.	Copper sulphate:	0.01M	in 100 ml distilled water.
2.	Sodium hydroxide:	2.5 M:	in 100 ml distilled water.
3.	Hydrogen peroxide.	6%	in 100ml distilled water.
4.	Sulphuric acid 3M		in 100 ml distilled water.
5.	p-dimethyl aminobenzaldehyde	5%	in 100 ml of propanol.
6.	Hydroxyproline standard	10mM	15 to 250mg/L.
7.	Potassium Hydroxide	12N	in 100 ml distilled water.
8.	Phenolphthalein reagent:	100 mg	in 100 ml of ethanol.

Note: Details regarding the precise volumes of specimens/ reagents and assay conditions, detection range etc., is provided in [**Table-1].**

Urinary Creatinine (Jaffe's Reaction):
Introductory Note:
Creatinine is a waste product formed in muscle by creatinine metabolism. Creatinine phosphate serves as storage form of energy in skeletal muscles. Creatine phosphate is spontaneously converted to creatinine and inorganic phosphate with release of high energy of 12.7Kcal. Creatinine formed is excreted in the urine. On a normal diet almost all creatinine in urine is endogenous. Its excretion is fairly constant from day to day and has been used to check the accuracy of 24 hours urine collection. It is independent of urine flow rate and its level in plasma is quite constant.

Principle:
Creatinine in alkaline medium reacts with picric acid to form a red colored tautomer complex of creatinine picrate; the concentration of the colored complex is directly proportional to serum creatinine. The absorbance measured at 520nm.

Chemicals / Reagents:
In-House prepared.

Reagents (Single Reagent Chemistry):
Reagent 1:
Sodium hydroxide 188mmol/L

Reagent 2:
Picric acid 50mmol/L

Preparation of Ready to use Reagent: Reagent 1 and Reagent 2 are mixed in 4:1 Ratio respectively, Working Reagent prepared freshly.

Note: Details regarding the precise volumes of specimens/ reagents and assay conditions, detection range etc., is provided in **[Table-1]**

Result and Discussion:-

Urinary excretion of Hydroxyproline is considered to be a useful indicator of changes in the metabolism of collagen because. Because collagen the much more abundant protein than any other protein containing hydroxyproline [13], [14]. On the other hand collagen comprises one-third of total body protein. Hence, urinary excretion of hydroxyproline justifies it relevance to measure the collagen turnover. The present study was carried out to assess the status of urinary hydroxyproline, creatinine and its derived parameter such as hydroxyproline: creatinine ratio [15], [16], [17]. The patients were recruited for the study at the Padmashree Diagnostics, Department of Gynecology, Vijayanagar, Bangalore, The study initiation started after obtaining the written informed consent. The study population consisted of 70 participants of premenopausal (n=35) and postmenopausal women (n=35), mean age of 38.11 ± 4.3 and 54.40 ± 4.6 respectively is suspected to be suffering from osteoporosis **[Fig-1], [Table-2]**.

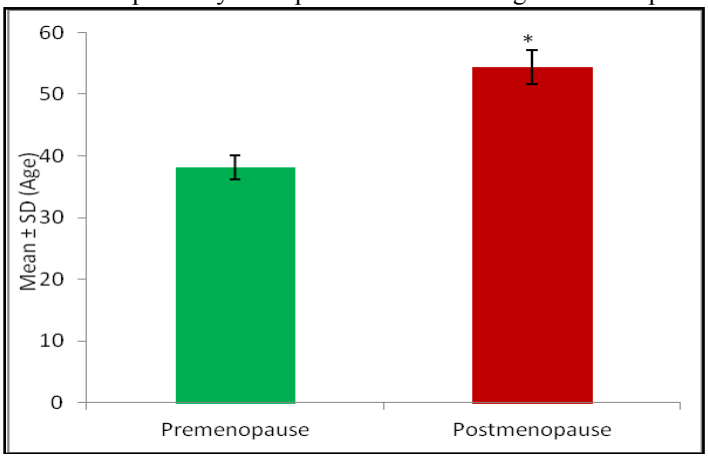

Fig1 :-Histogram represents the age distribution among the pre and post menopause women.Values expressed as Mean ± SD. Student's't' test: *: $p < 0.05$; **: $p < 0.01$: ***: $p < 0.001$.

Methodological Investigations:
The biochemical investigations such as urinary hydroxyproline and creatinine were studied. Considering the fact that establishing the normative data for osteoporosis suffering subjects is constrained by ethical clearance, the present study adopted an available reference interval from the literature. The details of the methodological aspect with respect to the compositions of the assay system and conditions are provided in **[Table-1]**.

Urine Hydroxyproline:
In recent years there has been increased attention on osteoporosis urinary biomarkers to evaluate the pre and post antiresorptive pharmacotherapy. Serum Vitamin D, urinary Calcium, Phosphorus, hydroxyproline and proline are the most often sought after investigations to confirm or to rule out the suspected Nutritional status in various inflammatory disorders.

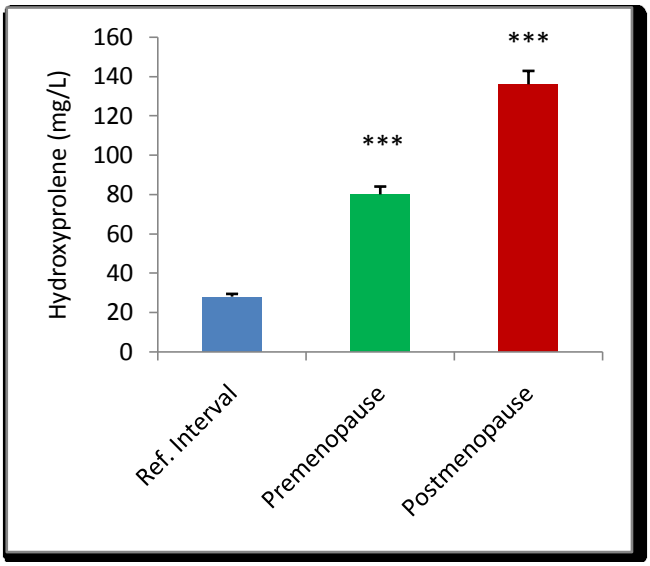

Fig-2 Histogram represents the urinary total Hydroxyproline in pre and post menopause women.Values expressed as Mean ± SD. Student's't' test: *: $p < 0.05$; **: $p < 0.01$: ***: $p < 0.001$.

Thus, in present study the bone mineralization marker urinary total hydroxyproline was quantified in pre and post menopausal woman. Study of this bone resorption marker will throw light for better understanding the pathophysiology of disease, as shown in histogram of pre and postmenopausal woman had 80.3 ± 75mg/L and 136 ± 103mg/L [**Fig-2**], **[Table-3]** respectively. Interestingly, the measured urinary hydroxyproline level in pre and postmenopausal group had significantly higher (***$p< 0.001$) than the normal reference interval. On the other hand, the excretion of hydroxyproline statistically significant in premenopausal woman but sill 41% hydroxyproline more excreted in postmenopausal woman. Further, to rule out any ambiguity in expressing urinary hydroxyproline level, the observed value was normalized to urinary creatinine excretion.

Urinary Creatinine:
It is important to monitor renal function to monitor various urinary biomarkers to rule out or to observe the manifestation by follow-up. By and large, biochemical investigations such as urea, creatinine, urinary creatinine clearance etc., explain the pathophysiology of the biochemical process. Substitution of urinary creatinine as a diagnostic /prognostic marker may helpful in dose fixing to the suboptimal level or to monitor possible drugs interactions affects on renal system [18]. It has also been considered as gold standard of practice for clinicians to normalize urinary excretion of many different biomarkers/abnormal metabolites to urine creatinine in chronic conditions such as microalbuminuria in diabetes mellitus and proteinuria in nephrotic syndromes [19]. Lack of normalization may lead to falsely low biomarker/abnormal metabolites concentration interpretation in glomerular filtration rate. In fact, spot assessments of urinary biomarkers of chronic kidney diseases normalized to urine creatinine concentrations have succeeded to replace 24 hrs urinary collections in many instances [20]. Thus, the

present study on urinary creatinine level in pre and post menopausal woman was 53.7 ± 14.2mg/dL and 37.0 ± 27.3 respectively [**Fig-3], [Table-3]**.

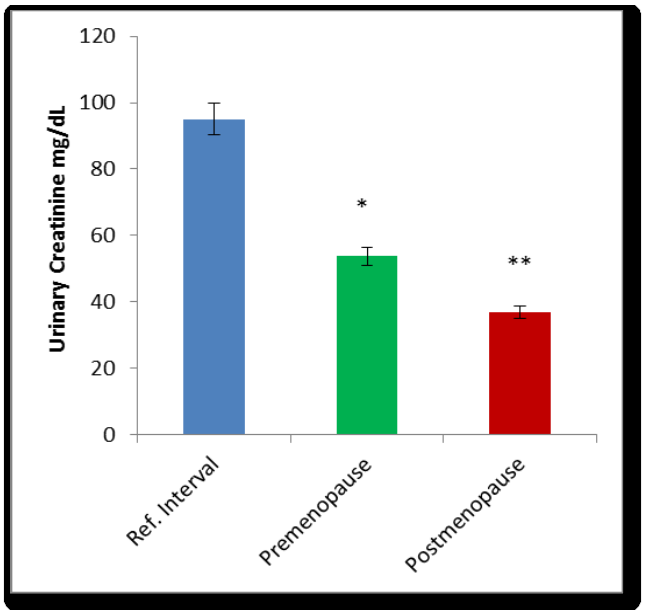

Fig-3 Histogram represents the urinary creatinine among the pre and post menopause women. Values expressed as Mean ± SD. Student's't' test: *: p <0.05; **: p<0.01: ***: p<0.001.

Hydroxyproline: Creatinine Ratio (ACR):

Among the constituent parameters of urinary abnormal metabolites are urine creatinine, urinary hydroxyproline and hydroxyproline **:** creatine ratio derived test parameter proves to be better diagnostic index among the urinary biochemical profiles. In particular, when the patient's urine hydroxyproline and urine creatinine level is ambiguous in their diagnostic relevance, i.e., being within the respective reference intervals, this particular derived test parameter provide better diagnostic index about the underlying pathophysiology of the diseases [21], [22].

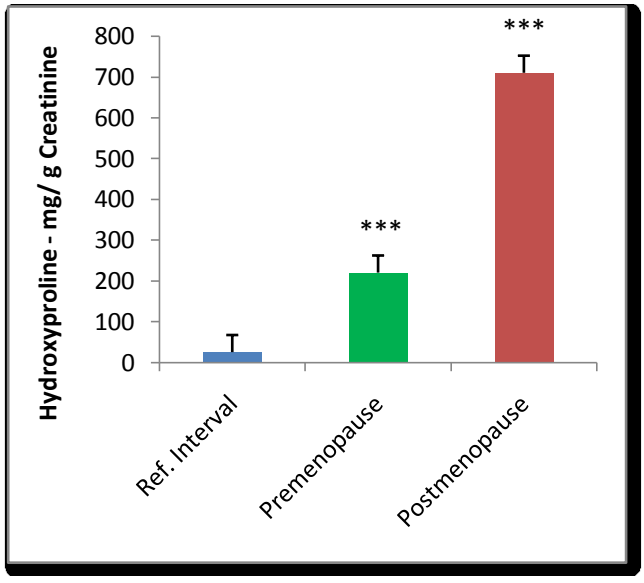

Fig-4 Histogram represents the Urinary Hydroxyproline: Creatinine Ratio among the pre and post menopause women. Values expressed as Mean ± SD. Student's't' test: *: p < 0.05; **: p < 0.01: ***: p < 0.001.

Thus, as shown in [**Fig-4]** the hydroxyproline: creatinine ratio (HCR) is a better diagnostic parameter as compared to the later urine parameter [**Fig-2]**. The diagnostic sensitivity of HCR is highly significant for most of the bone de/mineralization disorders like osteoporosis, Paget's disease, Bone tumorurolithiasis etc., as shown in [**Fig-2]**, by normalizing urinary excretion of hydroxyproline against urinary creatinine, in pre and postmenopausal group HCR degree of excretion ratio significantly changed from 41% to 69% against the normal reference interval(***$p<$ 0.001). The finding from these two test parameters such as urinary hydroxyproline and derived test parameter HCR imply that derived test parameter provide better diagnostic index about the underlying pathophysiology of the diseases. Thus, the normalized urinary total hydroxyproline excretion in pre and postmenopausal group was 220 ± 318mg/g Creatinine and 770 ± 928 mg/g Creatinine respectively.

Hence, while studying urine hydroxyproline, it is of relevance to relate the findings with urinary creatinine as well to evaluate the findings as to whether the observed changes in urinary total hydroxyproline would be a marker to elucidate the pathophysiology of post menopausal osteoporosis. Studying the total hydroxyproline biomarker is useful tool in monitoring the pharmacotherapy.

Summary and Conclusion:-

Descriptive Statistics of biochemical parameters:

The findings on descriptive statistics of various analyzed test parameters of serum and urine [Table-1] in pre and post menopausal woman of drug naïve cases studied as two group (n=35 each) with respective of age differences [Table-2] sub classified with respective reference intervals. Details regarding the precise volumes of specimens/ reagents and assay conditions, detection range etc., is provided in [Table-1]. In our current study, the distribution of data among pre and post menopausal group [Table-2] have shown significant difference between the groups and it underscores that studying urinary total hydroxyproline and its relevance to relate the finding with HCR is increased the diagnostic sensitivity to figure out the pathophysiology in post menopausal osteoporosis population [Table-3]. By and large, some of the salient findings of biochemical parameter like urinary hydroxyproline, creatinine, and the derived test parameter HCR are presented in the following sections as under summary and conclusions.

Summary :

Biochemical analysis of body fluids such as blood/serum/plasma and urine do provide a diagnostic window to evaluate the functional status of the quality of life. In case of pre and postmenopausal woman suspected to be suffering from osteoporosis disorders [23], [24], [25]. It has been shown that above mentioned parameters might play a significant role. However, our study also supports the view that increased excretion of total hydroxyproline content is a reflection of pathophysiology of diseases.

Hence, HCR should be considered as a risk factor in postmenopausal women suspected to be suffering from osteoporosis. Thus, the present randomized control, open-blinded and cross-sectional study was an attempt to further validate the routine chemistry parameters. Our study once again underscores the screening for HCR in apparently normal postmenopausal women may help us in identifying the individuals who are at increased risk for frequent bone fractures. The findings of this study, is an attempt to provide biochemical basis of this skeletal progressive metabolic disorder in pre and postmenopausal woman. It may be time to reevaluate our current concept of "normal" hydroxyproline excretion and to obtain a normal reference interval in Indian sub population by correlating the post menopausal urinary hydroxyproline with that of obtained normal reference range of premenopausal woman. In this context some of the salient feature of study outcomes is as follows.

1. It was that urinary hydroxyproline significantly increased in pre and post menopausal woman population when compared to available normal reference interval. **Fig-2**
2. By and large, the observed urinary creatinine level was found to be lower limit of reference interval in both the study group. Finding suggests that Creatinine elimination in the urine is significantly **[Fig-3]** decreased in both study groups.
3. These findings suggest that the prerequisite pharmacotherapy to reduce glomerular filtration pressure and to increase the creatinine excretion significantly. Findings also reiterate that urinary creatinine clearance could enhance the accuracy and credibility of drug clearance and increase the prognosis.
4. Among the urinary profile the derived parameter hydroxyproline: creatinine ratio (HCR)was found to be better diagnostic index. The measurement against to creatinine excretion rate has significantly increased the diagnostic sensitivity of hydroxyproline measurement.
5. The study of once again underscores that, HCR as a prognostic marker for antiresorptive pharmacotherapy outcomes in post menopausal woman. The current study states that comparative study between drug naïve pre

and post menopausal patient indicates the various risk factors such as low estrogen level, calcium and vitamin D deficiency and age related reduced calcium absorption leading to excess parathyroid hormone leading to bone resorption inIndian Sub population [**Fig-3], [Fig-4**]. But larger population based prospective studies on determinant factors of hydroxyproline excretion with other co-morbidity status are needed to be highly assessed. Hence, hydroxyproline: creatinine ratio can be one of the potential prognostic biomarkers for prospective studies.

Table-1:- Summary of automated biochemical assay settings and performance characteristics.

ASSAY PARAMETERS	URINARY HYDROXYPROLINE	URINARY CREATININE
Type of Specimens	Urine	Urine
Assay Method	End Point	End Point
Sample Volume (Normal)	1 ml (Hydrolysate)	15 µl
Sample Volume (Decrease) in µl	--	--
Sample Volume (Increase) in µl	--	--
Sample Dilution	Yes	Yes
Single Reagent Chemistry	--	Yes
R1 - $CuSO_4$	0.5 ml	750 µl
R2 - H_2O_2	0.5ml	--
NaOH	0.5ml	--
	Incubate for 5'min at 80^0C	--
H_2SO_4	2.0ml	--
P-dimethyl aminobenzaldehyde	1.0ml	--
Incubation	Incubate for 20'min at 70^0C	
Wavelength Primary (in nm)	540 nm	540 nm
Calibration Type	Linear	Linear
Calibrator (CRM) value	H54409	
Traceability of CRM	Sigma Aldrich	GC-MS
Multi Point (I - IV Stds)	-	-
Std I	15.87	-
Std II	31.75	-
Std III	62.5	-
Std IV	125	-
Std V	250	-
SD limit	0.05	0.1
Duplicate limit	15	100
Sensitivity limit	NA	2000
Abs limit (inc/dec)	Inc	Inc
Unit Expression	mg/L& mg/g Creatinine	mg/g of creatinine
Specific Characteristics of Assay		
Detection limit	15 - 250 mg/L	0.2 – 20 mg/dL
Reference Interval - Urine	28 ± 29.7	25 ± 7.2
Precision: Mean/ SD/ CV%		
Linearity: (Slope/ Correl/ Coeffi)	0.999 / 0.9996	1.024 / 0.998
Reagent Source	In- House prep	In-House prep
Instrument Used	Spectrophotometer	Spectrophotometer

Table-2:- Age distribution of pre and post menopause women.

SL No	Groups	Age in years		Unpaired t-test value
		Range	Mean ± SD	
1	Pre menopause	29-46	38.1 ± 4.3	* $p<0.05$
2	Post menopause	47-67	54.4 ± 4.6	

Table-3:- Urinary hydroxyproline and creatinine value in Pre and Post – menopausal women

SL No.	Parameter	Pre-menopause		Post-menopause		Unpaired t – test value
		Rang	Mean ± SD	Rang	Mean ± SD	
1	Urine Hydroxyproline	9-355	80 ± 75	4-3191	32±103.9	*** $p < 0.001$
2	mg/G Creatinine	78-1680	220 ± 318	80-1080	710 ± 924	*** $p < 0.001$

Table-4:- Reference ranges of assay parameters [19]

SL No.	Parameter	Reference range	Mean ± SD
1	Urine hydroxyproline	7-49 mg/24 hrs	28 ± 29.7
2	Mg/G creatinie	18-32mg/g	25 ± 7.2

Conclusion:-

To understand our findings, some characteristics of the present study need to be addressed. Our data were obtained between drug naïve pre and post menopausal woman from Indian sub population. The definition of pre menopausal urinary excretion rate of hydroxyproline level in Indian sub-population has been established. However, prospective collection of more number of pre menopausal urine samples enables to bring a normative data from premenopausal woman population. The obtained normative data for premenopausal woman population would be a new reference range in Indian sub-population or otherwise general population normative reference range commonly being used as reference interval in all kind of pathophysiological disorder. Hence, the derived parameter confirmed that HCR is the most prognostic significant diagnostic marker in pre and post menopausal patients. It could as well be considered as an indicator for therapeutic intervention by studying large scale pre and post menopausal woman population.

References:-

1. National Institute of Health.National Institute of Health State of the science Conference Statement:management of menopause related symptoms *Ann Intern Med* 2005:142 (12pt 1):1003-1013.
2. DM Vasudevan, Sreekumari S and KannanVaidyanathan. *Text Book of Biochemistry for Medical Students.* 7th edition. JAYPEE. 2013.
3. Llaneza P, FernándezIñarrea JM, Arnott B, GarcíaPortilla MP, Chedraui P, PérezLópez FR (2011). "Sexual function assessment in postmenopausal womenwith the 14item changes in sexual functioning questionnaire."*J Sex Med* 8 (8): 2144–51. doi:10.1111/j.17436109.2011.02309. (http://dx.doi.org/10.1111%2Fj.17436109.2011.02309. x). PMID 21679299 (https://www.ncbi.nlm.nih.gov/pubmed/21679299).
4. George BO. Urinary and anthropometrical indices of bonedensity in healthy Nigerian adults. J ApplSci Environ Mgt.2003;7(1):19–23
5. Ashumasachdeva, Shashiseth, AnjuHuria. Khosla, SumitSachadeva. Study of some common biochemical bone turn over markers in postmenopausal women . Indian journal of clinical Biochemistry, 2005,20(1)131-134.
6. Auth C, Seibel KJ, Robins SP, John P and Bilezikian (2008). Markers of bone metabolism. In: Beckers Text Book of Endocrinology Chapter 56, 3rd edition 548-557.
7. Sachdeva A, Seth S, Khosla AH and Sachdeva S (2005). Study of some common biochemical bone turnover markers in postmenopausal women. Indian Journal of Clinical Biochemistry 20(1) 131-134.
8. Brown JP et al, Canadian medical association journal, 2002 (5) 167-76.
9. Wayne W. Campbell and Minghua Tang. Protein Intake, Weight Loss, and Bone Mineral Density in Postmenopausal Women. Oxford Journals. The journals of Gerontology Series A: Biological Sciences and Medical Sciences. J Gerontol A BiolSci Med Sci. Oct 2010; 65A(10): 1115–1122. Published online Jul 6, 2010. doi: 10.1093/gerona/glq083.
10. Guyton, Hall. *Text Book of Medical Physiology.* 12th edition. Saunders Elsevier. 2011.
11. K Sembulingam, PremaSembulingam;*Essentials of Medical Physiology*; 6th Edition; JAYPEE Brothers Medical Publishers (P) LTD; 2012.
12. Neuman, R. E., and Logan, M. A. The determination of hydroxyproline. J. biol. Chem. 1950, 184, 299.
13. Cinnamon VanPutte, Jennifer regan, Andrew Russo, Rod Seeley, Trent Stephens, Philip Tate; *Seeley's Anatomy & Physiology*; 10th edition; New York; McGraw – Hill;
14. Malipatil BS, and ShilpaPatil; *Serum Electrolyte Status and Liver Functions in Different Phases of Menstrual Cycle*; Research Journal of Pharmaceutical, Biological and Chemical Sciences; 2013.*ISSN: 0975-8585.*

15. Gerard J. Tortora, Bryan Derrickson; *Introduction to the Human Body The Essentials od Anatomy and Physiology;* 9th edition; United States of America; john Wiley &Sons, Inc; 2012.
16. Kontturi, M. J., Sotaniemi, E. A., Larmi, T. K.I.: Hydroxyproline in the early diagnosis of bone metastases in prostatic cancer.Scand. J.Urol., 1974; 8, 91.
17. Laitinen, O.,Nikkila, E. A., Kivirikko, K. I.: Hydroxyproline in the serum and urine. Actamed. scand., 1966;179, 275.
18. Hodgkinson, T. Thompson Measurement of the fasting urinary hydroxyproline: creatinine ratio in normal adults and its variation with age and sex. J ClinPathol; 1982; 35:807-811.
19. Endres DB, Rude RK. Mineral and bone metabolism. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B. Saunders; 1998. 1395-14.
20. Calvo MS, Eyre DR, Gundberg CM. Molecular basis and clinical application of biological markers of bone turnover. Endocrin Rev 1996; 17: 333-68
21. Garnero P, Delmas PD. New developments in biochemical markers for osteoporosis. Calcification Tissue Int 1996; 59(Suppl 1): S2-S9.
22. Sachdeva A, Seth S, Khosla AH, Sachdeva S. Study ofsome common biochemical bone turnover markers in postmenopausal women. Indian J ClinBiochem 2005;20(1):131-134.
23. Sachdeva A, Seth S, Khosla AH, Sachdeva S. Study of some common biochemical bone turnover markers in postmenopausal women. Indian J ClinBiochem 2005; 20(1):131-134.
24. Fact and statistics about osteoporosis and its impact. International Osteoporosis Foundation, Osteoporosis Society of India. Action plan osteoporosis. Consensus statement of an expert group, New Delhi. 2011. http://www.osteofound.org/press_centre/fact_sheet.html, accessed on 12th December 2020.
25. Neetakumar, Ammini AC, Tandon N, Goswami R, Dineshkumar, Singh A. Ethnic variation of host and risk factors in silent epidemic of osteoporosis. Orthop Today. 2004;6(4):240–244.