VOLUME - 12, ISSUE - 12, DECEMBER - 2023 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper

Surgery

CLINICAL UTILITY OF SERUM LYSYLPYRIDINOLINE LEVELS IN THE DIAGNOSIS OF DIABETIC FOOT OSTEOMYELITIS – A COMPARATIVE CROSS-SECTIONAL ANALYTICAL STUDY.

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ABSTRACT

Background: The current diagnostic methods for Diabetic foot osteomyelitis have significant concerns, including invasiveness(biopsy), extended lag-time to positivity(radiograph) and poor sensitivity. Lysylpyridinoline is a serum bone turnover marker released during bone resorption. It has high sensitivity and specificity. Early detection of diabetic foot osteomyelitis using serum Lysylpyridinoline levels may reduce the need for amputation. **Aims and objectives:** To assess diagnostic utility of serum Lysylpyridinoline(LP) levels in diabetic foot osteomyelitis. **Method:** The study was conducted as cross sectional, comparative analytical study from April 2019 to January 2021. Patients who had positive bone to probe test and bone biopsy specimen culture were included in osteomyelitis group(DFO). Patients who had negative results in both were included in diabetic foot ulcer group(DFU). Serum Lysylpyridinoline level was compared between the groups. **Results:** A total of 120 participants were included in the study, 60 in each group. Mean corrected serum LP levels were higher in osteomyelitis (5.113±1.93 ng/ml) than DFU group (4.03±1.47 ng/ml) and the difference was statistically significant($P<0.05$). Receiver Operating characteristic curve(ROC) was plotted for demonstrating its diagnostic value. The area under the curve was 0.733(95%CI: 0.643-0.824) and found to be statistically significant. The cut off value was 3.959 ng/ml with sensitivity of 81.7%, specificity of 75.56% to differentiate between DFU with and without osteomyelitis. **Conclusion:** Serum Lysylpyridinoline levels were significantly higher in patients with diabetic foot osteomyelitis patients. Hence can be used for screening and diagnosing of diabetic foot osteomyelitis.

KEYWORDS : Bone biopsy, Lysylpyridinoline, Screening, Osteomyelitis.

INTRODUCTION:

Diabetic foot osteomyelitis(DFO) occurs in 20- 50% of all diabetic patients depending on its severity, and its chronicity contributes to a poor quality of life associated with pain, suffering and disability[1]. Diabetic foot bone infection (osteomyelitis) is a leading cause of hospitalization and lower limb amputation worldwide[2,3]. The current diagnostic methods for osteomyelitis each have significant concerns, including invasiveness (biopsy), extended lag-time to positivity (radiograph), poor sensitivity (plain radiograph) and poor specificity (bone scan). Leukocyte scans, bone scans, MRIs, and biopsies all require specialized skills and are costly and have limited availability in resource-poor regions.[4,5]

Lysylpyridinoline(LP) is a serum bone turn over marker released during bone resorption[5,6]. Studies have shown that even a small volume of bone generally affected in diabetic foot osteomyelitis would raise serum LP[6]. The sensitivity, specificity, positive predictive value and negative predictive value of LP for the diagnosis of diabetic foot osteomyelitis were 100%, 77.8%, 89.0% and 100%, respectively[6]. LP in the diagnosis of diabetic foot osteomyelitis may reduce the need for sophisticated diagnostic imaging and early detection of osteomyelitis. It can be more useful as a triaging test to assess for further evaluation with specialized diagnostic imaging. To the best of our knowledge only one study is available regarding significance of LP levels in diabetic foot osteomyelitis[6]. The study aims to assess the diagnostic utility of serum Lysylpyridinoline levels in the diagnosis of diabetic foot osteomyelitis by comparing its levels in diabetic foot patients with and without osteomyelitis.

METHODOLOGY:

This study was conducted as a cross-sectional comparative analytical study for diagnostic test accuracy, from April 2019 to January 2021 in the department of General Surgery in a tertiary care centre. The study was approved by the Institute Ethics committee. All patients more than 18 years of age with Diabetic foot infections were recruited in the study. Diabetic patients with known osteomyelitis unrelated to a diabetic foot ulcer, Charcot's joint, any metabolic bone or joint diseases, bone cancer or bone metastasis, organ failure, on treatment with thiazolidinedione, or immunosuppressive drugs were excluded.

All patients with diabetic foot ulcer / cellulitis underwent probe to bone test(PTB) to look for risk of underlying osteomyelitis. Patients who had PTB positive(hard stop/ underlying bone felt) underwent percutaneous bone biopsy specimen culture using a bone biopsy needle (11G) from the underlying bone. Patients who had positive bone biopsy culture were categorized as Diabetic foot osteomyelitis(DFO) group. Patients who had negative PTB and negative bone biopsy culture were categorized as Diabetic foot ulcer without osteomyelitis(DFU). Demographic characteristics, Diabetes disease characteristics and Details of the diabetic foot infection were collected from both groups. All patients in both groups underwent diabetic workup- the following tests were performed for all participants: Blood sugar profile, HbA1c, urine routine microscopy, ECHO and Xray of the local part standard procedure. Along with routine sampling, an extra 5ml blood sample was collected for Lysylpyridinoline assay from group A and group B participants. The concentration of serum Lysylpyridinilone levels was estimated using the ELISA method. Levels of serum Lysylpyridinilone in both groups was compared.

The sample size was calculated with an expected area under the curve (AUC) for the serum Lysylpyridinoline levels for diagnosing diabetic foot osteomyelitis as 0.65 at a 5% level of significance and 90% power. The estimated sample size was 120 with 60 patients in each group

Statistical analysis

Data were analysed using SPSS version 19.0. The results were explained in the form of baseline identification and the participants' socioeconomic parameters in terms of descriptive and inferential statistics. The descriptive statistics include frequencies, proportions, and percentages for categorical variables, while the continuous variables were

analysed and explained in terms of mean and standard deviation. Other measures of central tendency and variation like median were analysed wherever applicable. The inferential statistics were explained based on the statistical test's application to find out the significant difference in proportions by Chi- square test. For continuous variables in the form of scores, a significant mean score with other essential parameters was done either by t-test or Mann-Whitney U test. The receiver operating characteristic (ROC) curve was plotted for serum Lysylpyridinilone levels and the area under the curve (AUC) along with 95 % CI was calculated. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were also calculated along with 95% CI.

RESULTS:

A total of 120 patients were included in this study, with 60 patients each in diabetic foot ulcer without osteomyelitis and diabetic foot ulcer with osteomyelitis. The mean age in DFO was 56.18 ± 10.57 years, while the mean age in the DFU group was 54.35 ± 11.40 years. Both groups had male predominance, 49(81.7%) patients in the DFU group and 44(73.3%) patients in the DFO group. The mean duration of diabetes in the DFO group was 5.40±4.8 years, and in the DFU group was 4.24 ± 4.4 years. This difference was not found to be statistically significant (P >0.05)(Table.1)

Table.1: Comparison of demographic and clinical parameters in the study population.

PARAMETERS		DFO	DFU	p-value
Mean age ± S.D (in years)		56.18±10.5	54.35±11.4	
Mean duration of DM ± S.D (in years)		5.4±4.8	4.2±4.4	0.09
Duration of ulcer (in months)	<1 month	4	54	0.001
	>1 month	17	6	
Area of the ulcer(cm)	<20 cm	18	23	0.13
	>20 cm	42	18	
Depth of ulcer (cm)	<2 cm2	0	34	0.001
	>2 cm2	60	26	
Wagner's grade	<2	0	8	0.001
	>2	60	2	
PTB test	Positive	60	16	0.001
	Negative	0	25	
X – ray changes	Present	18	0	0.001
	Absent	42	60	

The mean duration of ulcers in the DFO group was 81.5±293.77 days, and in the DFU group, it was 19.33±26.85 days. This difference was found to be statistically significant (P <0.05). 31.7%(N=19) in the DFU group had cellulitis with intact skin. 24(40%) cases in the DFO group had an ulcer size of 21-50 cm^2 range. 23(38.3%) cases in DFU group had less than 20 cm^2. The mean area of the ulcer in the DFO group was 43.21±44.78cm^2, whereas, in the DFU group, it was 36.46±56.47 cm^2. This difference was not found to be statistically significant (P-value >0.05). All the cases in the DFO group had a deep ulcer(>2cm) as compared to 26(43.3%) cases in the DFU group. This difference was found to be statistically significant (P-value <0.05). All DFO group cases either belonged to Wagner's grade 3 or more (100.0%), out of which toe gangrene was present in 7(11.7%) cases. 96.7% of the cases in DFU belonged to ≤2 Wagner's grading. This difference was found to be statistically significant (P-value <0.05).(Table.1)

19 patients out of 120 subjects had cellulitis with intact skin. Out of the rest (N=101), 84 patients tested positive for PTB test. All the DFO group subjects had tested positive for PTBT, whereas 40%(N=24) in DFU tested positive for PTBT. This difference was found to be statistically significant (P-value <0.05). 18(30%) cases in the DFO group had X-ray features of OM, whereas 42(70%) subjects had no features suggestive of osteomyelitis in X-ray. All the patients in DFU had no X-ray features suggestive of osteomyelitis. This difference was found to be statistically significant (P-value <0.05). (Table.1)

Mean RBS at the time of admission in the DFO group was 270.66±123.04 mg/dl, and in the DFU group, it was 240.68±122.83 mg/dl. Mean HbA1C in the DFO group was 9.035±2.12%, and in the DFU group, it was 9.423±2.66%. The mean total protein in the DFO group was 5.127±0.66 g/dl, and in the DFU group, it was 6.740±0.68 g/dl. Mean albumin in the DFO group was 2.210±0.48 g/dl, and in the DFU group it was 2.497±0.49 g/dl. Total protein and albumin had significant statistical difference between two groups (P-value <0.05). Mean corrected serum LP levels in DFO group was 5.113±1.93 ng/ml, and in DFU group was 4.032±1.47 ng/ml. This difference was found to be statistically significant (P<0.05).(Table.2)

Table.2: Comparison of blood and biochemical parameters in the study population.

Parameters		DFO	DFU	p-value
RBS(mg/dl)	<180	16	24	0.203
	>180	44	36	
HbA1c	<6	1	5	0.379
	>6	59	55	
TLC	<7000	2	2	0.475
	>7000	58	58	
Total Protein(g/dl)	<6	55	7	0.001
	>6	5	53	
Albumin(g/dl)	<2.5	48	34	0.28
	>2.5	12	26	
Protein corrected LP levels(ng/ml)		5.11	4.03	0.001

Receiver Operator Characteristic curve of corrected serum Lysylpyridinoline levels in the diagnosis of diabetic foot osteomyelitis, was plotted. The optimum cut off was 3.959 ng/ml which had an area under the curve of 0.733 (95% CI: 0.643 – 0.824), sensitivity of 81.7%, specificity of 56.7%, positive predictive value of 65.33% and negative predictive value of 75.56% to differentiate between DFU with and without osteomyelitis. (Figure.1)

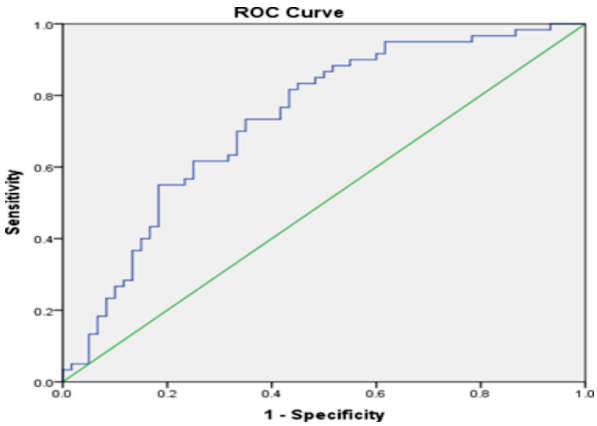

Figure.1. Receiver operator characteristics of corrected serum Lysylpyridinoline levels in the diagnosis of diabetic foot osteomyelitis.

A sub group analysis was done for the DFO group to compare

the diagnostic value of X- ray and PTBT as compared to the gold standard bone biopsy specimen culture. Table.3 shows the diagnostic value of a probe to bone test and x-ray compared to bone biopsy specimen culture in the study population. 18 subjects out of 60 in the DFO group had osteomyelitis features in X-ray, while none out of 60 had osteomyelitis features in the non-osteomyelitis group. X-ray compared to bone biopsy specimen culture had 30% sensitivity, 100% specificity, positive predictive value of 100% and negative predictive value of 58.82%. 84 out of 101 were positive for the probe to bone test, but 24 were excluded as not having osteomyelitis group because they were tested negative for bone biopsy specimen culture. Probe to bone test compared to bone biopsy specimen culture had 100% sensitivity, 60% specificity, positive predictive value of 71.43% and negative predictive value of 100%.

Table.3. Diagnostic value of PTB, X-ray and serum Lysylpyridinoline levels in comparison to bone biopsy specimen culture in the study population.

	PTB	X-RAY	Serum LP levels
Sensitivity	100%	30%	81.7%
Specificity	60.98%	100%	56.7%
Positive predictive value	78.95%	100%	65.33%
Negative predictive value	100%	58.82%	75.56%

DISCUSSION:

Diabetic foot osteomyelitis is a medical, therapeutic and diagnostic problem, and many of the condition's outcomes are linked to late diagnosis, insufficient or ineffective care. Major or minor amputation is the most severe complication of diabetic foot osteomyelitis. Major amputation is linked to a substantial decrease in these patient's life expectancy. Due to non-availability in rural/remote and underserved healthcare facilities, current diagnostic modalities for DFO have substantial disadvantages. At present, there is no low-cost diagnostic modality available with higher sensitivity and specificity in diagnosing diabetic foot osteomyelitis.

Various theories were proposed on the role of serum bone turnover markers in the diagnosis of osteomyelitis. In our study we found that the Serum Lysylpyridinoline(LP) were significantly higher in patients with diabetic foot osteomyelitis as compared to diabetic foot ulcer patients. Diagnostic utility of Serum LP was also studied and we demonstrated that serum LP levels can be used for screening of diabetic foot osteomyelitis.

In our study, risk factors for osteomyelitis were being analysed. The mean age of the study population was 55.26 years with major proportion constituted by Males (77.5%). Patients who developed osteomyelitis had a longer duration of diabetes (5.4 years). In addition, they had, elevated HbA1c levels and higher RBS levels. Clinical characteristics like greater depth of ulcer (>2cm) and with larger area (>4cm^2) were found to have higher chance of developing osteomyelitis. Patients with osteomyelitis were found to have lower protein and albumin levels (5.12±0.66 and 2.21±0.48). Studies done in Poland (Kulwas et al. 2017)[7], Pondicherry (Elamurugan et al.2010)[8] Pondicherry (Praveen et al. 2017)[9], UAE (Manda et al. 2012)[10] Chandigarh (Seth et al. 2019)[11] have identified similar risk factors for osteomyelitis.

In our study, 30% of the patients in the diabetic foot ulcer with osteomyelitis group had features detectable in X-ray. X-ray in our study has shown 30% sensitivity, 100% specificity, positive predictive value of 100% and negative predictive value of 58.82% compared to bone biopsy specimen culture. The higher sensitivity noted in our study can be related to more profound infections and chronicity of ulcer, which would have given an adequate period for the bone changes to occur and become detectable on plain radiograph.

A study conducted by Hayes et al from Australia in 2016 noted that patients with diabetic foot osteomyelitis had elevated serum Lysylpyridinoline levels[6]. The results were similar to current study. We found that patients with diabetic osteomyelitis had serum LP levels of 5.11±1.9ng/ml, which was significantly higher than without osteomyelitis (4.03±1.4ng/ml). The cut off value was 3.95ng/ml had the maximum AUC. Patients with diabetic osteomyelitis had low protein levels. Hence a corrected serum LP levels were calculated in the study. Diagnostic value of serum LP was calculated and compared with gold standard method bone biopsy. The sensitivity, specificity, positive and negative predictive value were 81.7%,56.7%, 65.33% and 75.56% respectively. The Study conducted by Hayes et al, had a higher sensitivity of 100% when compared to our study[6]. This dissimilarity might be because of their study's smaller sample size.

Our study has few limitations. Usage of serum biomarkers in clinical practice is still relatively new. In addition, serum LP levels is affected by multiple factors which affect bone metabolism including poor glucose control, patients on haemodialysis etc. Despite these limitations, the potential value of lysylpyridinoline(LP) is particularly intriguing because it has never been used before, particularly in resource-constrained rural health centres where modern diagnostic facilities are scarce and, when available, are often out of reach of the average patient.

CONCLUSION

Serum lysylpyridinoline (LP) levels are significantly higher in patients with diabetic foot osteomyelitis as compared to diabetic foot ulcer patients. Our findings show that serum LP levels can be used for screening and diagnosing diabetic foot osteomyelitis.

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