ORIGINAL RESEARCH PAPER
Volume - 13 | Issue - 11 | November - 2024 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

SERUM MMP 9 AND hsCRP – MOLECULAR GUIDE FOR GRAFTING IN DIABETIC FOOT ULCER

Medical Science

Dr. S. Prasanna Balaji* MD, Assistant Professor, Department Of Biochemistry, Government Medical College, Nagapattinam. *Corresponding Author

Dr. R. Vasanthi MD, Assistant Professor, Department Of Biochemistry, Tirunelveli Medical College, Tirunelveli.

Dr. R. Shanmugapriya MD, Assistant Professor, Department Of Biochemistry, Thiruvarur Medical College, Thiruvarur.

Dr. Dhanalakshmi Murugan MBBS, Second Year Postgraduate, Department Of General Medicine, Shri Muthukumaran Medical College Hospital And Research Institute, Chennai.

ABSTRACT

Summary: Diabetic foot ulcers (DFU) are prime cause for 85 % of limb amputation in diabetes patients. Triggered by uncontrolled blood sugar, Internal milieu of diabetic patients exhibit a state of chronic inflammation. There is uninhibited increase in levels of extracellular matrix degrading proteinases like Matrix Metallo Proteinase 9, marked by high levels of serum C Reactive Protein. Hence, wound healing is delayed for years. Skin grafting is one modality of treating chronic diabetic foot ulcers. Although the prerequisite for grafting remains the same for all diabetic foot ulcers, certain wounds show failed skin graft uptake. Hence, apart from routine wound assessment, there is a dire need for a molecular guide to predict successful outcomes. So, we evaluated the inflammatory markers MMP9 and hs-CRP levels serially in diabetic patients with foot ulcers on the day of admission, preoperative day and 5th postoperative day after grafting. **Results:** Biochemical parameters were analyzed on day of admission, day before and five days after grafting. Wound with successful graft uptake showed a 29% reduction in serum MMP 9 levels and 28% reduction in serum hs-CRP levels on the preoperative day compared with admission. **Conclusion:** MMP-9 Is a mediator of the phagocytic NADPH oxidase. High MMP 9 results in chronic inflammatory state. Decrease in serum MMP9 levels mark reduction in inflammation, which aids in extracellular matrix growth. Hence, along with routine wound assessment, serial monitoring of MMP 9 and hs-CRP levels becomes key for successful timing of skin grafts in diabetic foot ulcers and successful outcomes.

KEYWORDS

Serum MMP 9, Diabetic Foot, Wound Grafting, Molecular guide for healing

INTRODUCTION

With increasing prevalence of diabetes, incidence of complications has increased. Lifetime risk of developing foot ulcer for diabetics is 15%[1]. In India, the prevalence of diabetic foot ulcers is 3.6%[2]. Socio-cultural practices such as barefoot walking, walking on fire, use of improper footwear, poor knowledge regarding foot care - attribute to increased prevalence of foot complications in India[3]. Diabetic foot is often challenging to treat owing to compromised healing, higher risk of infection and higher risk of lower-limb amputation[4]. Expedited closure of diabetic foot wounds can reduce the risk of major limb amputation and decrease costs. Split-thickness skin grafts are the ideal choice, because of shorter healing times and lower cost compared with other wound-healing modalities[5]. Despite adequate local conditions (no infection, no ischemia, adequate offloading, good granulation tissue), 20% of dermal grafts did not integrate with wound bed 6. MMP 9 is a matrix modulator and plays crucial role during inflammatory phase of wound healing. However, high MMP 9 levels are associated with lower wound closure rates[6]. Inflammatory markers like MMP 9, hs-CRP could influence the integration of dermal grafts despite euglycemic status as inflammation is antecedent event in diabetes. Our goal was to provide surgeons with a molecular marker that could predict the outcome of the grafting for a better management of diabetic foot ulcer.

MATERIALS AND METHODS

It is a prospective longitudinal study and comprised of 40 subjects

Inclusion Criteria:

Type 2 diabetes mellitus patients with foot ulcer for more than six months.

Exclusion Criteria:

- Foot ulcers with osteomyelitis
- Foot ulcers with exposed bone, tendon
- Foot ulcers with complications (infection, angiopathy)
- Patients with cardiovascular disease, hypertension, bronchial asthma and rheumatoid arthritis.

Study was approved by institutional human ethical committee. Written informed consent was obtained from all patients before the study. Study was conducted for the period of 6 years from January 2018 to December 2023. Blood samples were collected three times during the study i.e. on the day of admission, on the preoperative day and on the 5th post operative day. All the samples were stored at -80° till the analysis.

Baseline Investigations-

Complete hemogram, Fasting blood glucose, Urea, Creatinine, HbA1c, total Cholesterol, Tri acyl glycerol (TGL), HDL and VLDL cholesterol were analysed. LDL was calculated using Fridewald formula.

Levels of Matrix metalloproteinase- 9 (MMP-9), hs-CRP and insulin were assayed using ELISA kits. One way ANOVA was done using Minitab 18 and p value less than 0.05 was considered significant.

Table 1: General Patient Characteristics

PARAMETER	STUDY GROUP (MEAN± SD)
Age	59.32 ± 7.60
Duration of diabetes(yrs)	14.63 ± 5.75
Duration of ulcer(months)	12.25 ± 6.17
WHR	0.95 ± 0.09
WBC	7859.90 ± 1980.25
Urea (mg/dl)	28.26 ± 6.07
Creatinine (mg/dl)	0.97 ± 0.16
Uric acid (mg/dl)	4.65 ± 0.88
HbA1C (%)	7.50 ± 0.85
Total cholesterol (mg/dl)	188.80 ± 26.55
Serum triglycerides (mg/dl)	150.31 ± 53.5
HDL (mg/dl)	48.96 ± 8.73
LDL (mg/dl)	106.90 ± 25.05
VLDL (mg/dl)	30.11 ± 10.79

Data are mean ± SD.

Table 3: One Way ANOVA

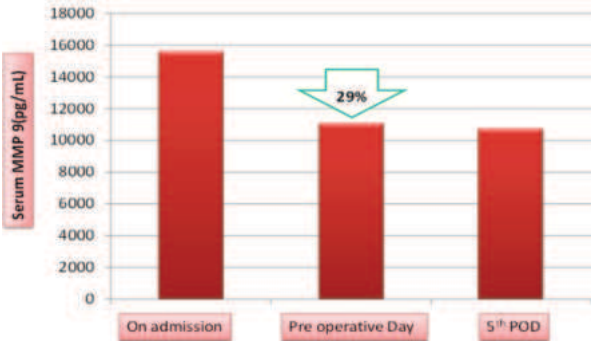
PARAMETER	ONE WAY ANOVA
FBS (mg/dl)	P<0.001
PPBS (mg/dl)	P<0.001

SERUM MMP9 (pg/ml)	P<0.001
INSULIN (IU/ml)	P<0.001
hs-CRP (mg/L)	P<0.001

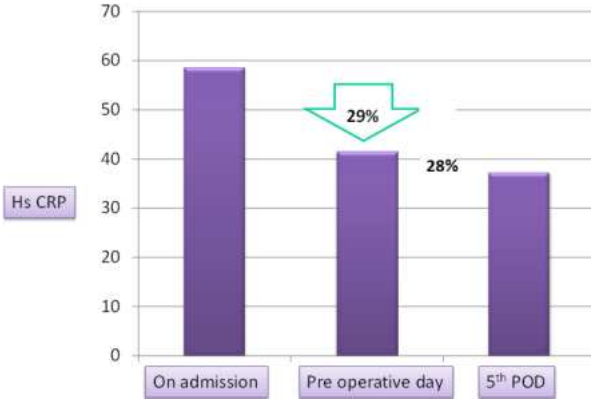
Table 2: Biochemical Parameters Assessed On Admission, Preoperative Day And 5TH Postoperative Day (POD)

PARAMETER	ON ADMISSION I	PREOPERATIVE DAY II	5TH POD III
FBS(mg/dl)	199.15 ± 35.80	108.30 ± 12.59	107.03 ± 11.27
PPBS(mg/dl)	290.67 ± 62.26	144.90 ± 18.02	141.65 ± 16.64
SERUM MMP9 (pg/ml)	15615.60 ± 987	11068 ± 1116	10726 ± 1128
INSULIN (IU/ml)	55.66 ± 4.60	38.43± 5.05	37.60 ± 4.74
hs-CRP(mg/L)	58.63 ± 7.21	41.53 ± 5.84	37.30 ± 4.82

Data are mean ± SD.



Graph 1: Serum MMP 9 Levels On Admission, Pre-operative Day, 5TH POD



Graph 2 : Serum Hs-crp Levels On Admission, Pre-operative Day And 5 TH POD

DISCUSSION

Type 2 diabetes mellitus is characterized by insulin resistance and hyperglycaemia. Foot ulcers precede 50% to 70% of lower limb amputations in diabetes patients[1]. Hyperglycaemia and associated inflammation are major causative factors in development of such complications[7]. Accelerated inflammation due to hyper active immune system is antecedent event in diabetes mellitus[8]. Macrophage infiltration of pancreatic Islet is an early event in Type 2 Diabetes mellitus. It precedes 8 weeks before the onset of disease in mice fed with high-fat diet[9]. In a vicious cycle, Inflammation precedes insulin resistance and insulin resistance aggravates inflammation. Over the last century, Insulin Resistance was also found in chronic inflammatory states like obesity, arthritis, Systemic Lupus Erythematosus, ankylosing spondylitis alongside with psychiatric illnesses like schizophrenia & depression[8]. Matrix Metallo Proteinase 9 (MMP 9) is a gelatinase that acts primarily on type IV collagen, elastin, fibronectin and Interleukin 1b[10]. A delicate balance overlies synthesis of MMP 9. Under expression of MMP 9 affects normal wound healing, while over expression leads to chronic non healing wounds, owing to its matrix degrading Property.

Though hs-CRP is considered as an acute phase reactant, several studies indicate its importance for predicting inflammatory status in diabetic patients[11,12,13]. Hence in chronic inflammatory state like diabetes mellitus, hypertension there is long term elevation of hs-CRP levels. In diabetes Mellitus patients, Insulin resistance up regulates MMP 9 promoter and mRNA, leading to Matrix Metallo proteinase 9 over synthesis[14]. Also, Insulin resistance promotes NADPH oxidase over activity in phagocytes, which increases free radical production15. High oxidative stress up-regulates MMP-9. Persistently elevated MMP 9 levels attribute to chronicity of ulcers, despite tight glycaemic control and culture negative wound.

Figure 1: On The Day Of Admission

Figure2: Day Before Grafting

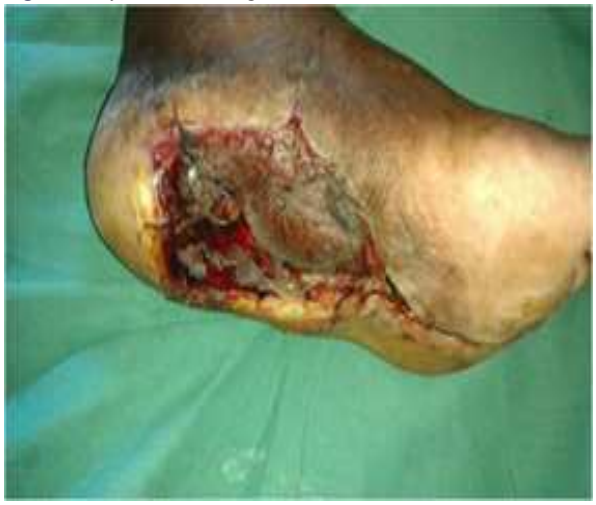


Figure 3: FIVE DAYS AFTER GRAFTING

Valentina et al in 2014 observed that high MMP 9 levels are associated with dermal graft failures in diabetic foot ulcers[6].

In our study, we observed successful graft uptake in all the forty patients. There was a 29% decrease of MMP9 and 28 % decrease of hsCRP levels on preoperative day in comparison with levels on admission day. Liu et al in 2014 described that increased MMP9 in wound fluid predicts poor wound healing in diabetic foot ulcers. This could also explain graft failure despite the presence of tight glycaemic control, culture negative wound and absence of other complications. Hence monitoring serum MMP 9 levels during the course of grafting could help in predicting the outcome.

CONCLUSION

Serum MMP 9, an inflammatory marker could aid in proper timing of grafting and help in preventing graft failure. Monitoring MMP 9 and hs – CRP levels in patients with diabetic foot ulcers slated for skin grafting will help deciding the ideal time for the procedure and hence enabling successful outcomes reducing the costs associated with it.

REFERENCES

1. Leone S, Pascale R, Vitale M, Esposito S. [Epidemiology of diabetic foot]. Infez Med 2012; 20 Suppl 1: 8-13 [PMID: 22982692]
2. Ramsey SD, Newton K, Blough D, et al. Incidence, outcomes, and cost of foot ulcers in patients with diabetes. Diabetes Care 1999;22(3):382–7
3. Vijay V, Snehalatha C, Ramachandran A. Sociocultural practices that may affect the development of the diabetic foot. IDF Bull. 1997;42:10-12.
4. Rastogi.A, Bhansali.A.Diabetic Foot Infection: An Indian Scenario.J Foot Ankle Surg (Asia-Pacific) 2016;3(2):71-79
5. Impact of Diabetes and Comorbidities on Split-Thickness Skin Grafts for Foot Wounds. Journal of the American Podiatric Medical Association _ Vol 103 _ No 3 _ May/June 2013.
6. Valentina Izzo, Marco Meloni et al. High Matrix Metalloproteinase Levels Are Associated With Dermal Graft Failure in Diabetic Foot Ulcers. The International Journal of Lower Extremity Wounds 2014, Vol. 13(3) 191– 196.
7. Kaul K, Hodgkinson A, Tarr JM, Kohner EM, Chibber R Review Is inflammation a common retinal-renal-nerve pathogenic link in diabetes?.Curr Diabetes Rev. 2010 Sep; 6(5):294-303
8. Rainer H Straub : Insulin resistance, selfish brain, and selfish immune system: an evolutionarily positively selected program used in chronic inflammatory diseases Arthritis Research & Therapy 2014, 16(Suppl 2):S4
9. Ehses JA, Perren A, Eppler E et al. Increased number of islet-associated macrophages in type 2 diabetes. Diabetes 2007; 56: 2356–2370.
10. T. Klein R. Bischoff Physiology and pathophysiology of matrix metalloproteases Amino Acids (2011) 41:271–290
11. Maria Fizelova, Raimo Jauhiainen et al.(2017) Differential associations of inflammatory markers with insulin sensitivity and secretion: the prospective METSIM study. The Journal of Clinical Endocrinology & Metabolism Endocrine Society. DOI: 10.1210/jc.2017-01057
12. Ridker P M C-reactive protein and the prediction of cardiovascular events among those at intermediate risk: moving an inflammatory hypothesis toward consensus. J Am Coll Cardiol 2007; 49:2129-2138.
13. Saltevo J, Laakso M, Jokelainen J, Keinanen-Kiukaanniemi S, Kumpusalo E, Vanhala Levels of adiponectin, C-reactive protein and interleukin-1 receptor antagonist are associated with insulin sensitivity: a population-based study. Diabetes Metab Res Rev 2008; 24:378-383.
14. A. P. Yu, B. T. Tam, W. Y. Yau, K. S. Chan, S. S. Yu, T. L. Chung and P. M. Siu* Yu et al. Association of endothelin 1 and matrix metallopeptidase 9 with metabolic syndrome in middle aged and older adults . Diabetol Metab Syn.dr (2015) 7:111 DOI 10.1186/s13098-015-0108-2
15. Ana Fortunoa, Julen Bidegaina, Gorka San Jose :Insulin resistance determines phagocytic nicotinamide adenine dinucleotide phosphate oxidase overactivation in metabolic syndrome patients. 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins DOI:10.1097/HJH.0b013e3283