

## Association of Serum MMP 9 Levels With Insulin Resistance in Diabetic Patients with Foot Ulcers before and After Grafting

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### I. Introduction

Diabetes mellitus is a glooming threat worldwide. It amounted for around 9% of deaths in the year 2012, in Southeast Asia<sup>1</sup>. India stands second among world countries with whopping estimate of 69.2 million cases out of 415 million cases worldwide<sup>2</sup>. Chronic hyperglycemia leads to sensory and autonomic neuropathy, peripheral vascular disease, nephropathy and retinopathy.

Amongst all the complications of diabetes, the prevalence of diabetic foot infections is most common affecting approximately 15% of the diabetic patients<sup>3</sup>. This can be attributed to several social and cultural practices such as barefoot walking, inadequate facilities for diabetes care and education, and poor socioeconomic conditions<sup>4</sup>.several studies have proven that, at molecular level chronic hyperglycemia coupled with excessive up regulation of extracellular matrix degrading gelatinases like MMP 9 could be the root cause of all these complications. Primary pathology here vests with insulin resistance. Hence in this study we evaluated the relationship between serum MMP 9 and insulin resistance in patients with diabetic foot ulcers before and after skin grafting .

### II. Materials And Methods

Study comprised of 40 subjects with diabetic foot ulcers.

#### INCLUSION CRTIERIA:

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

#### EXCLUSION CRITERIA:

Patients with hypertension, bronchial asthma, rheumatoid arthritis and cardiovascular complications.

Patients with diabetic foot complications like infection, osteomyelitis.

Ethical clearance was obtained from institutional human ethics clearance committee. Fasting blood samples were obtained from the patients three times during the course of treatment ie on the day of admission, preoperative day and 5<sup>th</sup> post operative day. Fasting blood glucose, Urea, Creatinine, HbA1c, total Cholesterol, Triglycerides, HDL cholesterol, LDL cholesterol and VLDL cholesterol. Complete haemogram were analyzed.

Levels of Matrix metalloproteinase-9 (MMP-9) and insulin were measured using enzyme- linked immunosorbent assay kits.

Statistical analysis Data was analyzed using Statistical Package for Social Science (SPSS).Results are expressed as mean±SD for normally distributed data. One way ANOVA was used for MMP 9, INSULIN and HOMA-IR. P-value less than 0.05 was considered to be statistically 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

**Table 2:** special parameters

PARAMETER	ON ADMISSION	PREOPERATIVE DAY	5 <sup>TH</sup> POD
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
Serum INSULIN (µIU/ml)	55.66 ± 4.60	38.43 ± 5.05	37.60 ± 4.74
HOMA IR	29.96 ± 5.81	10.33 ± 1.93	9.93 ± 1.63

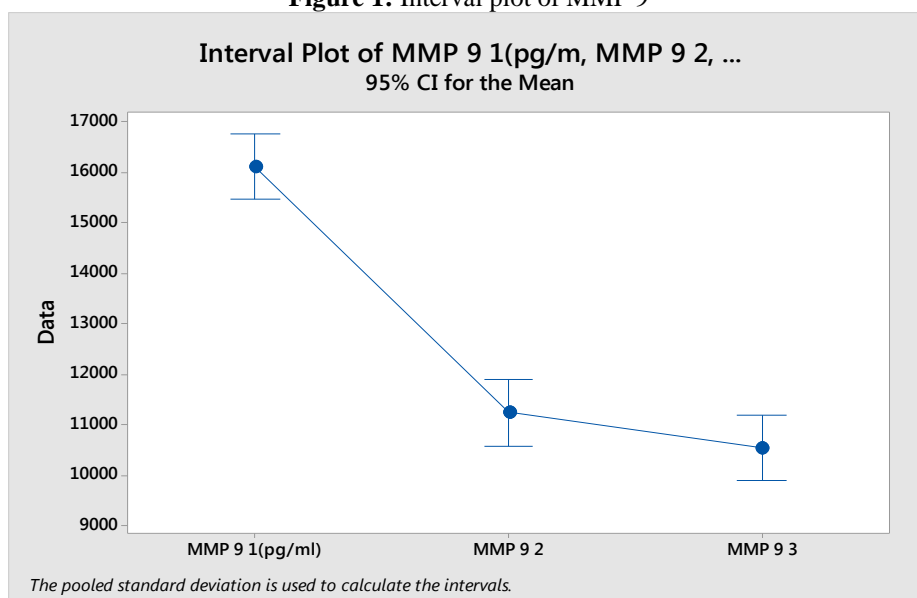
Data are mean±SD.

**Table 3 :** one way ANOVA

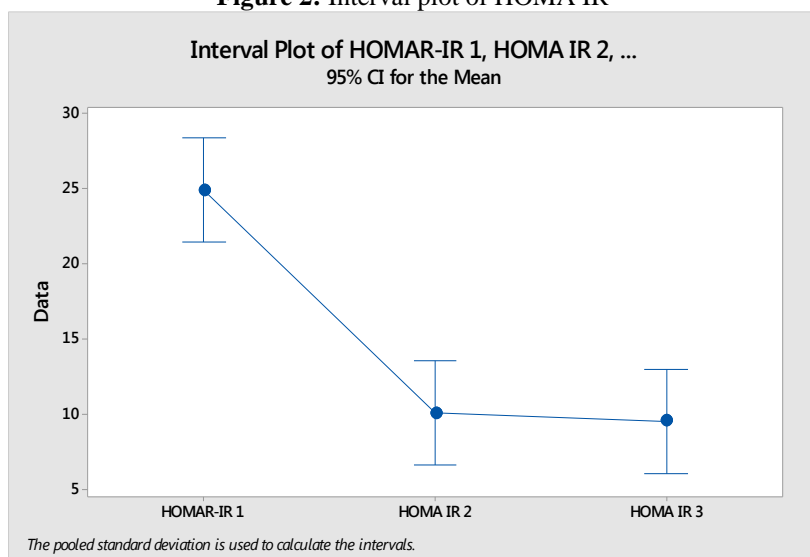
P – value	1 vs 2	1 vs 3	2 vs 3
FBS	<0.001	<0.001	<0.001
PPBS	<0.001	<0.001	<0.001
Serum MMP 9	<0.001	<0.001	<0.001
Serum INSULIN (µIU/ml)	<0.001	<0.001	<0.001
HOMA IR	<0.001	<0.001	<0.001

1 = on admission; 2 = on preoperative day; 3 = on 5<sup>th</sup> POD

**Figure 1:** Interval plot of MMP 9



**Figure 2: Interval plot of HOMA IR**



### III. Discussion

Foot ulcer is devastating complication of diabetes mellitus, which affects 15% of diabetic patients during their lifetime<sup>5</sup>. Apart from neuropathy and peripheral vascular disease, the chronicity of DFU is attributed to insulin resistance and elevated levels of serum Matrix Metallo proteinase 9 (MMP 9) along with profound oxidative stress.

Optimum healing of cutaneous wound entails a well orchestrated cohesion of complex molecular events of cell migration and proliferation and of extra cellular matrix deposition and remodeling. There must be appropriate and precise cellular response to inflammatory mediators, growth factors and cytokines. This orderly process of healing becomes flawed in diabetic foot ulcers. A wide array of pathognomic abnormalities ranging from disease specific intrinsic flaws in blood supply, angiogenesis and matrix turn over to extrinsic factors due to neuropathy and continued trauma contributes to failure to heal.

Normal wound healing occurs in three phases. (1) Inflammatory phase – where phagocytosis occurs (2) proliferative phase – where angiogenesis, collagen deposition, granulation tissue formation, epithelialization and wound contraction occurs. Fibroblasts and keratinocyte play a vital role during this stage.(3) Remodeling phase – collagen is realigned along tension lines<sup>6</sup>.

While acute wounds progress through the different phases of wound healing, the healing process in diabetic patients becomes stalled resulting in chronic non-healing wounds<sup>7</sup>. The inflammatory phase is prolonged, but is ineffective as macrophages have impaired efferocytosis, showing decreased competence to phagocytose cells, and also neutrophils show reduced chemotactic and phagocytic activities<sup>8</sup>.In addition, ischemia and vascular disease reduces healing capacity of the wound<sup>8</sup>. Defective angiogenesis is, in part caused by the impaired expression of VEGF and higher oxidative stress<sup>9</sup>.

Besides regulating glucose and lipid metabolism, insulin is involved in protein synthesis, mitochondrial biogenesis, cellular growth, proliferation, differentiation, and migration<sup>10</sup>.In experimental mouse models ,Insulin stimulates the proliferation of keratinocyte and production of matrix proteins including fibronectin, collagen and various proteoglycans; there by facilitating healing process<sup>11</sup>.Moreover, skin fibroblasts involved in the development of granulation tissue, are known to express Insulin Receptor and their growth rate is increased by insulin<sup>12</sup>.In DFU, Primary pathology that initiates this complication, stems from insulin resistance. Hence insulin resistance leads to prolonged inflammatory phase during wound healing that ultimately leads to diabetic foot ulcers.

Poor glycemic control owing to Insulin resistance leads to chronic hyperglycemia, that inturn promotes up regulation of MMP 9 promoter and mRNA that ultimately leads to inappropriate increase in levels of matrix Metallo proteinase 9<sup>13</sup>. Matrix Metallo Proteinases (MMPs) are zinc containing endopeptidases capable of degrading all components of the extracellular matrix (ECM)<sup>14</sup>. Insulin resistance is associated with NADPH oxidase over activity in phagocytes that increases free radical production<sup>15</sup>. Oxidative stress alters the wound micro environment and delays wound healing. High oxidative stress up-regulates MMP-9, which inturn increases the levels of angiostatin – which is a potent anti-angiogenic and it antagonizes the effect of VEGF.

Paresh dandona et al have observed than intravenous infusion of insulin causes acute lowering of serum MMP 9 levels<sup>16</sup>. Thus insulin regulates MMP 9 levels both directly and indirectly.

At the time of admission, the biochemical evaluation showed poor glycemic control with elevated insulin & MMP 9 levels. On improvement of glycemic status, the wound bed showed progressive increase in granulation tissue. On the preoperative day, there was a significant decrease in levels of MMP 9 and insulin.

As the glycemic status of the patient improves, insulin secretion decreases by feedback mechanism. With good glycemic control, MMP 9 levels decreases. This is evidenced by granulation tissue growth at wound site. Since the wound under goes a phase of rapid healing after grafting the levels of MMP 9 attains a plateau. Improving insulin resistance is pivotal in treating DFUs.

#### IV. Conclusion

Insulin regulates the function of epidermal and dermal skin cells, vascular as well as immune cells, all of which are important factors in the skin's healing response. Associating serum MMP 9 levels with insulin resistance gives better insights in to wound microenvironment. Overcoming insulin resistance and finding out an effective way to deliver insulin to wound site is the biggest puzzle before us. This opens multiple doorways for cost effective treatment modalities and for improving quality of life of patients with DFUs.

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<sup>5</sup> WJD 5th Anniversary Special Issues (4): Diabetes-related complications

<sup>6</sup> ROBINS TEXT BOOK OF PATHOLOGY: 236;6<sup>th</sup> edition

<sup>7</sup> Tellechea A, Kafanas A, Leal EC et al (2013) Increased skin inflammation and blood vessel density in human and experimental diabetes. *Int J Low Extrem Wounds* 12:4-11

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<sup>15</sup> Insulin resistance determines phagocytic nicotinamide adenine dinucleotide phosphate oxidase overactivation in metabolic syndrome patients .Ana Fortunoa, Julen Bidegaina, Gorka San Jose´ a et al. 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins DOI:10.1097/HJH.0b013e3283

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