Oxidative Stress and Antioxidant Status Before and after Grafting in Diabetic Patients with Foot Ulcer

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ABSTRACT

Introduction: Diabetic foot ulcer (DFU) is the third most common complication and it is estimated that 85% of lower limb amputation in diabetes patients are preceded by foot ulcers. Oxidative stress plays a significant role in regulating normal wound healing by facilitating hemostasis, inflammation, wound closure, and development and maturation of the extracellular matrix. So we evaluated oxidative stress and antioxidant status in diabetic patients with foot ulcers before and after grafting.

Material and methods: It was a prospective longitudinal study in Rajah Muthiah medical college, chidambram, and comprised of 40 subjects. Written informed consent was obtained from all patients before the study. 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 -80o till the analysis.

Results: Biochemical parameters were analyzed on day of admission, day before and five days after grafting. There was a significant reduction of MDA but there was no significant change in antioxidant status.

Conclusion: In diabetic patients with chronic non healing wound, lymphocyte apoptosis is initiated by the augmentation of reactive oxygen species which leads to the increased expression of proapoptotic proteins and decreased expression of antiapoptotic proteins. But there was no change in antioxidant status. Reduced oxidative stress could be due to reduced inflammation. To conclude, there was reduction of oxidative stress after grafting which would help in wound healing in diabetic foot ulcer.

Keywords: Oxidative Stress, Antioxidant, Grafting, Diabetic Patients, Foot Ulcer

INTRODUCTION

Diabetic foot ulcer (DFU) is the third most common complication and it is estimated that 85% of lower limb amputation in diabetes patients are preceded by foot ulcers. Owing to lack of knowledge and awareness regarding the disease and its complications, incidence of both disease and complication has steadily increased. Inflammation is the root cause of diabetes. This up regulates oxidative stress on one hand. On the other hand, hyperglycemia and it related consequences increase oxidative stress.[1-3] Owing to increased oxidative stress, the extra cellular matrix is altered and this delay wound healing. In concurrence with earlier studies, we evaluated oxidative stress and antioxidant status in diabetic patients with foot ulcers before and after grafting. Oxidative stress was measured as TBARS (thiobarbutric acid reacting substances) and antioxidant status was measured as FRAP (ferric reducing antioxidant power).This could provide better insights about the role of oxidative stress and antioxidants during the course of healing.

MATERIAL AND METHODS

It was a prospective longitudinal study in Rajah Muthiah medical college, chidambram, and comprised of 40 subjects. Study was approved by institutional human ethical committee. Written informed consent was obtained from all patients before the study. 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^{o} till the analysis.

Inclusion crtieria

Type 2 diabetes mellitus patients with foot ulcer for more than six months (figure 1,2,3).

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.

Baseline investigations- Complete haemogram, Fasting blood glucose, Urea, Creatinine, HbA1c, total Cholesterol, Tri acyl glycerol (TGL), HDL and VLDL cholesterol were analyzed. LDL was calculated using Fridewald formula. Levels of Matrix metalloproteinase-9 (MMP-9) and insulin were assayed using ELISA kits. TBARS and FRAP was assayed used chemical method.

STATISTICAL ANALYSIS

One way ANOVA was done using Minitab 18 and p value less than 0.05 was considered significant.

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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-1: General patient characteristics

Parameter	On admission	Preoperative day	5TH 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
FRAP (moles/L)	2260.93 ± 491.57	2330 ± 503.93	2370.90 ± 515.82
TBARS (µmoles/L)	4.16 ± 1.32	3.57 ± 1.28	3.08 ± 1.23
Data are mean ± SD			

Table-2: Biochemical parameters assessed on admission, preoperative day and 5th postoperative day (pod)

FBS(mg/dl)	P<0.001
PPBS(mg/dl)	P<0.001
SERUM MMP9 (pg/ml)	P<0.001
FRAP (moles/L)	P<0.001
TBARS (moles/L)	P<0.001

Table-3: One way ANOVA

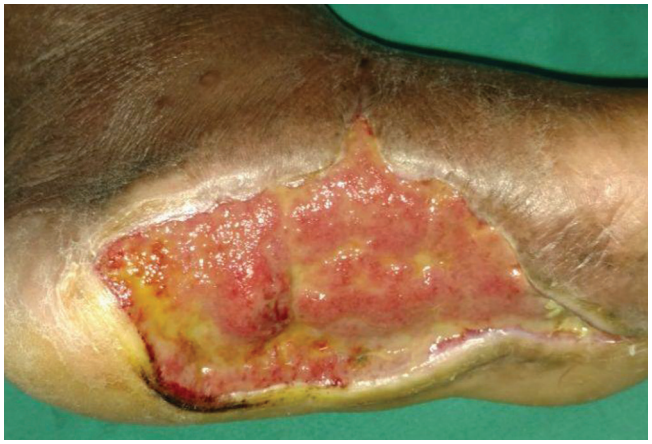

Figure-1: Diabetic foot – on the day of admission (wound bed filled with necrotic slough)

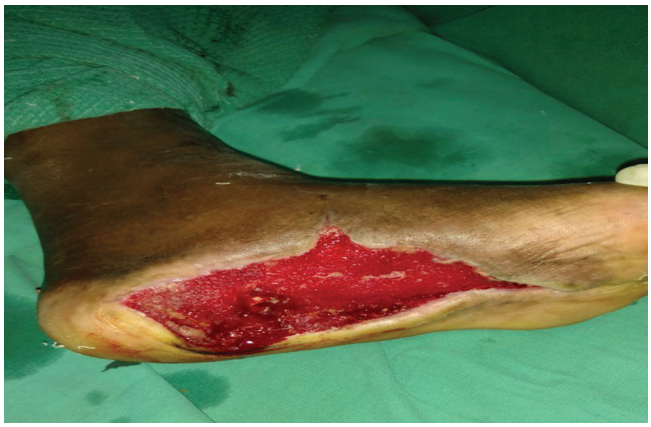

Figure-2: Day before grafting (wound bed filled with granulation tissue)

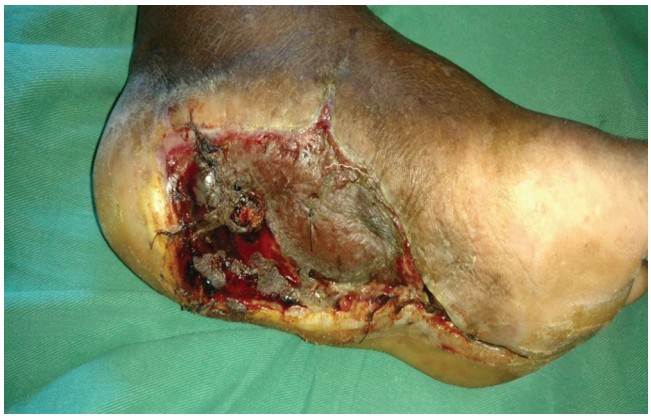

Figure-3: five days after grafting (well taken graft)

DISCUSSION

Diabetes mellitus accounts for 425 million cases worldwide. India ranks second with 72.9 million cases.[1] Diabetes kills and disables, striking people at their most productive age impoverishing families, reducing the life expectancy of older people worldwide. Diabetes is a common threat that does not respect borders or social class. The burden of diabetes drains national healthcare budgets, reduces productivity, slows economic growth, causes catastrophic expenditure for vulnerable households and overwhelms healthcare systems.

Diabetes mellitus is a pro-Inflammatory state predisposing to many complications like atherosclerosis, retinopathy, neuropathy and nephropathy. Peripheral neuropathy is the most common form of diabetic neuropathy which affects the distal nerves of the limbs, particularly those of the feet, accounting for 6.4% of all diabetes related complications.[1] It alters mainly the sensory function,causing progressive numbness, which facilitates the development of ulcers (diabetic foot) because of external trauma. 85% of lower limb amputations in diabetes patients are preceded by foot ulcers.[1] The prevalence of diabetic foot is higher among people with type 2 diabetes, compared to people with type 1 diabetes.[2]

Free radicals are produced as a consequence of biological oxidation in mitochondria, which is a part of normal metabolism. Oxygen metabolism always produces oxygen-derived free radicals such as superoxide (O2•-), hydroxyl (OH•), alkoxyl (RO•), peroxyl (RO2•), peroxynitrite (ONOO-) and oxygen derived non-radicals such as hydrogen peroxide (H2O2), hypochlorous acid (HOCl) and hypobromous acid (HOBr). At low levels, free radicals exert beneficial effects on cellular responses and immune function. As a part of host defense mechanism, Phagocytes release free radicals to destroy invading pathogens.[3] In granulomatous diseases like sarcoidosis,where there is a defective membrane bound NADPH oxidase system and defective production of superoxide anion radical, incidence of multiple and persistent infections is high. They also regulate intracellular signaling cascade in non phagocytic cells like fibroblasts, thyroid follicles, and vascular smooth muscle cells.[4]

The production of these free radicals and its physiological effects are counter balanced by cellular antioxidants. Antioxidants like reduced glutathione (GSH), superoxide dismutase (SOD) and catalase protects cells against oxidative damages. Anti oxidants scavenge these free radicals. There exists a delicate balance between the two. When this balance is lost, that is when these free radicals are produced in excess; they exert a phenomenon called as oxidative stress.

Type 2 diabetes mellitus is characterized by insulin resistance and consequent hyperglycemia. Hyperglycemia triggers chronic Inflammation, with concomitant elevated levels of reactive oxygen species.[5] Moreover oxidative stress induces insulin resistance by inducing GLUT 4 polymorphism. GLUT 4 is considered to be central in determining peripheral insulin sensitivity. Nava Basha et al in 2005 have demonstrated that disruption of a single allele of the GLUT4 gene resulted in a T2DM-like phenotype, whereas over expression of GLUT4 protected against the development of diabetes in an animal model.

In T2DM patients with chronic non healing wound, there is increased expression of proapoptotic proteins like Caspases, FAS, BAX and decreased expression of antiapoptotic proteins like B-cell lymphoma 2 genes (Bcl-2).[6] In streptozotocin-induced diabetic rats, the elevated blood sugar level increases cellular apoptosis.[7]

Redox signaling is a key regulator of wound healing, especially through its influence on extracellular matrix (ECM). Aberrant redox signaling and increased oxidative stress are widely accepted contributors to the development of diabetic foot ulcer.

In contrast to normal healing, wound healing in diabetes is uncoordinated and spatiotemporally disorganized. Diabetic wounds are characterized by prolonged inflammatory phase, a limited proliferation phase, and irregular remodeling. Since the concentration of antioxidant enzymes is very low in intracellular space, extra cellular matrix is prone for oxidative damage even under normal circumstances.[8] Oxidative stress in diabetes exacerbates this further.

Oxidation of cysteine and methionine in the protein side chains, forms protein adducts, which interfere with protein structure and function.[9] Free radicals significantly damage glycosaminoglycans present in the ECM.[9] Collagen oxidation induces inappropriate cross linking and disrupts its triple helical structure.[10]

ROS also mediate AGE accumulation in the ECM.[11] Elevated ROS in diabetes favor the formation of AGE by inhibiting Glyceraldehyde 3 phosphate dehydrogenase activity, and causing the accumulation of glyceradehyde-3-phosphate (G3P), a glycolysis intermediate. G3P can be non enzymatically converted to methylglyoxal, a highly reactive and abundant intracellular AGE precursor.[12] ROS also contribute the formation of AGE through both glycation and oxidation reactions. The glycoxidation products further glycate collagen, elastin, and fibronectin in the ECM.[13] Like direct oxidative damage to the ECM, glycation alters mechanical proteins and cellular interactions.

In our study we evaluated the antioxidant status through FRAP and oxidative stress through T-BARS. On the day of admission, the biochemical investigations showed high blood glucose levels with concomitant elevated oxidative stress. When the wound showed signs of healing and is considered fit for grafting, there was a significant reduction in TBARS along with good glycemic status. Five days after grafting TBARS levels attained a plateau. Antioxidant levels as measured by FRAP remained almost constant throughout the course of treatment.

CONCLUSION

Oxidative stress disturbs the ECM formation and delays wound healing in diabetes mellitus patients. With good glycemic control, oxidative stress is reduced, it results in better wound healing and improved insulin sensitivity. Reduction of oxidative stress by supplementing antioxidants needs to be explored by further studies

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