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ASSOCIATION OF CYTOCHROME P450 MONO-OXYGENASE AND NEUTROPHIL MPO (MYELOPEROXIDASE) ACTIVITY IN DIABETES AND NON-DIABETIC FOOT ULCER PATIENTS

KEY WORDS: Cytochrome P450 mono-oxygenase, Neutrophil MPO, Diabetes, non-diabetic foot ulcer.

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ABSTRACT

Diabetic foot ulcer is a major complication of diabetes mellitus, and probably the major component of the diabetic foot. The aim of the study to find any association of Diabetic foot ulcer with Cytochrome P450 mono-oxygenase and Neutrophil MPO(Myeloperoxides) Activity

We found that activation of Cytochrome p450 Mono Oxygenase level was significantly lower in Type2 DM with Foot Ulcer than Healthy Control (t=8.5553). T-test showed that mean Cytochrome p450 Mono Oxygenase of Type2 DM with Foot Ulcer patients had significantly lower than others. Over expression of Neutrophil Myeloperoxidase level was significantly higher in Type2 DM with Foot Ulcer than Healthy Control (t=57.7285). T-test showed that mean Neutrophil Myeloperoxidase of Type2 DM with Foot Ulcer patients had significantly higher than others.

Adverse Foot Ulcer events associated with Type II diabetes may be in part a result of enhanced Cytochrome p450 Mono Oxygenase expression and activity.

INTRODUCTION

Diabetic foot ulcer is a major complication of diabetes mellitus, and probably the major component of the diabetic foot.

Wound healing is an innate mechanism of action that works reliably most of the time. A key feature of wound healing is stepwise repair of lost extracellular matrix (ECM) that forms the largest component of the dermal skin layer.[1]But in some cases, certain disorders or physiological insult disturbs the wound healing process. Diabetes mellitus is one such metabolic disorder that impedes the normal steps of the wound healing process. Many studies show a prolonged inflammatory phase in diabetic wounds, which causes a delay in the formation of mature granulation tissue and a parallel reduction in wound tensile strength.[1]

Treatment of diabetic foot ulcers should include: blood sugar control, removal of dead tissue from the wound, wound dressings, and removing pressure from the wound through techniques such as total contact casting. Surgery in some cases may improve outcomes.[2] Hyperbaric oxygen therapy may also help but is expensive.[2]

It occurs in 15% of people with diabetes,[3] and precedes 84% of all diabetes-related lower-leg amputations.[4]

In order for a wound to heal, extracellular matrix not only needs to be laid down but also must be able to undergo degradation and remodeling to form a mature tissue with appropriate tensile strength.[5] Proteases, namely matrix metalloproteinases are known to degrade almost all the extracellular matrix components. They are known to be involved in fibroblast and keratinocyte migration, tissue re-organization, inflammation and remodeling of the wounded tissue.[1,5] Due to persistently high concentrations of pro-inflammatory cytokines in diabetic ulcers, MMP activity is known to increase by 30 fold when compared to acute wound healing.[6] MMP-2 and MMP-9 show sustained overexpression in chronic non-healing diabetic ulcers.[1,7] Balance in the MMP activity is usually achieved by tissue inhibitor of metalloproteinases (TIMP). Rather than absolute concentrations of either two, it is the ratio of MMP and TIMP that maintains the proteolytic balance and this ratio is found to be disturbed in diabetic ulcer.[8,9] In spite of these findings, the exact mechanism responsible for increased MMP activity in diabetes is not known yet. One possible line of thought considers Transforming growth factor beta (TGF-) as an active player. Most MMP genes have TGF- inhibitory element in their promoter regions and thus TGF– regulates the expression of both MMP and their inhibitor TIMP.[10] In addition to the importance of cell-cell and cell-matrix interactions, all phases of wound healing are controlled by a wide variety of different growth factors and cytokines. To mention precisely, growth factors promote switching of early inflammatory phase to the granulation tissue formation. Decrease in growth factors responsible for tissue repair such as TGF- is documented in diabetic wounds. Thus, reduced levels of TGF in diabetes cases lower down the effect of inhibitory regulatory effect on MMP genes and thus cause MMPs to over express.[3,11,12]

The balance between matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) is crucial for normal wound healing processes. A low MMP/TIMP ratio is a good predictor of successful wound-healing in diabetic foot ulcers. Diabetes creates an unfavorable ratio. It increases the activity and expression of MMP-9, MMP-2, and MMP-8 while reducing TIMP-2 . The abnormally elevated level of MMPs may impair cell migration and result in sustained inflammation with net increased tissue destruction. In the chronic diabetic foot lesions, local administration of protease inhibitors reduces the ratio of MMP/TIMP and improves wound healing.[13]

The aim of the study was to find any association of Diabetic foot ulcer with Cytochrome P450 mono-oxygenase and Neutrophil MPO(Myeloperoxides) Activity

MATERIALS AND METHODS

INCLUSION CRITERIA

1. Vascular foot ulcers
2. Neuropathic foot ulcers
3. Infective foot ulcers
4. Healthy Control.

EXCLUSION CRITERIA

1. Traumatic Ulcers
2. Steroid Induced Ulcers
3. Malignant Ulcers
4. Radiation Ulcers
5. Skin diseases

SAMPLE DESIGN

1. Healthy Control, 50
2. Diabetic population with foot ulcer, 50
3. Diabetic population without foot ulcer, 50
4. Non-diabetic population with foot ulcer, 50

Study group:

1. Healthy Control, 50 persons
2. Diabetic population with foot ulcer, 50patients
3. Diabetic population without foot ulcer, 50patients
4. Non-diabetic population with foot ulcer, 50patients

STATISTICAL ANALYSIS:

For statistical analysis data were entered into a Microsoft excel spreadsheet and then analyzed by SPSS (version 24.0; SPSS Inc., Chicago, IL, USA) and GraphPad Prism version 5. Data had been summarized as mean and standard deviation for numerical variables and count and percentages for categorical variables. One-way analysis of variance (one-way ANOVA) was a technique used to compare means of three or more samples for numerical data (using the F distribution). A chi-squared test ($\chi 2$ test) was any statistical hypothesis test wherein the sampling distribution of the test statistic is a chi-squared distribution when the null hypothesis is true. Without other qualification, 'chi-squared test' often is used as short for Pearson's chi-squared test. Unpaired proportions were compared by Chi-square test or Fischer's exact test, as appropriate. p-value ≤ 0.05 was considered for statistically significant.

NEUTROPHIL MYELOPEROXIDASE AND CYTOCHROME-P450- ESTIMATED ELISA METHOD

Myeloperoxidase (MPO) Activity Assay Kit ab105136 is a rapid, simple, sensitive, and reliable colorimetric assay suitable for use as a high throughput MPO activity assay.

In the MPO assay protocol, myeloperoxidase produces HClO from H_2O_2 and Cl-. The HClO reacts with taurine to generate the taurine chloramine, which subsequently reacts with the DTNB probe to eliminate color (absorbance at 412 nm). The absorbance is inversely proportional to the amount of MPO enzyme.
Neutrophil myeloperoxidase and cytochrome- p450 were measured by standard ELISA methods

RESULT

We found that in mean age was higher in type2 DM with foot ulcer patients than others and that had statistically significant (p<0.0001).In type2 DM with foot ulcer, male patients was significantly higher (p=.00138). In non-diabetic foot ulcer, female patients were significantly higher (p< .00001). In type2 DM without foot ulcer, female patients was significantly higher (p=.00006). In healthy control, female patients was significantly higher (p=.00032).In non-diabetic foot ulcer, house wife patients were significantly higher (p< .00001). In type2 DM without foot ulcer, house wife patients was significantly higher (p=.00006). In healthy control, house wife patients was significantly higher (p=.00032).

In type2 DM with foot ulcer, the mean Cytochrome p450 Mono Oxygenase(mean±s.d.) of patients was 18.4800 ± 2.0726. In non-diabetic foot ulcer, the mean Cytochrome p450 Mono Oxygenase(mean±s.d.) of patients was 25.8600 ± 6.2727. In type2 DM without foot ulcer, the mean Cytochrome p450 Mono Oxygenase(mean±s.d.) of patients was 24.2400 ± 3.5084. In Healthy Control, the mean Cytochrome p450 Mono Oxygenase(mean±s.d.) of patients was 26.3000 ± 6.1221. Distribution of mean Cytochrome p450 Mono Oxygenase vs. group was statistically significant (p<0.0001).

In type2 DM with foot ulcer, the mean Neutrophil Myeloperoxidase (mean±s.d.) of patients was 401.6000 ± 22.0213.In non-diabetic foot ulcer, the mean Neutrophil MPO (mean±s.d.) of patients was 254.5000 ± 11.8773.In type2 DM without foot ulcer, the mean Neutrophil Myeloperoxidase (mean±s.d.) of patients was 330.9000 ± 15.6391.In Healthy Control, the mean Neutrophil Myeloperoxidase (mean±s.d.) of patients was 160.0400 ± 19.7618.Distribution of mean Neutrophil Myeloperoxidase vs. group was statistically significant (p<0.0001).

Table 1: Distribution of mean Cytochrome p450 Mono Oxygenase

	Group	Number	Mean	SD	Minimum	Maximum	Median	p-value
Cytochrome p450 Mono Oxygenase	Type2 DM with foot ulcer	50	18.4800	2.0726	15.0000	22.0000	19.0000	<0.0001
	Non diabetic foot ulcer	50	25.8600	6.2727	16.0000	45.0000	25.0000	
	Type2 DM without foot ulcer	50	24.2400	3.5084	20.0000	35.0000	24.0000	
	Healthy Control	50	26.3000	6.1221	20.0000	48.0000	25.0000	

	T Statistic	P-value
Type2 DM with Foot Ulcer vs. Healthy Control	8.5553	<0.0001
Type2 DM without Foot Ulcer vs. Healthy Control	2.0644	0.0416
Non diabetic foot ulcer vs. Healthy Control	0.3550	0.7234

Table 2: Distribution of mean Neutrophil Myeloperoxidase

	Group	Number	Mean	SD	Minimum	Maximum	Median	p-value
Neutrophil Myeloperoxidase	Type2 DM with foot ulcer	50	401.6000	22.0213	270.0000	420.0000	408.0000	<0.0001
	Non diabetic foot ulcer	50	254.5000	11.8773	240.0000	270.0000	250.0000	
	Type2 DM without foot ulcer	50	330.9000	15.6391	310.0000	350.0000	330.0000	
	Healthy Control	50	160.0400	19.7618	145.0000	280.0000	158.0000	

	T Statistic	P-value
Type2 DM with Foot Ulcer vs. Healthy Control	57.7285	<0.0001
Type2 DM without Foot Ulcer vs. Healthy Control	47.9403	<0.0001
Non diabetic foot ulcer vs. Healthy Control	28.9695	<0.0001

DISCUSSION

Diabetic foot ulcer is the common dreadful complication of diabetes mellitus. The lifetime prevalence of foot ulceration is about 15%. [14] Macro and microvascular involvement and neuropathy plays a major role in the pathophysiology of diabetic foot ulcers. [15] According to the Diabetes Atlas 2013 published by the International Diabetes Federation, the number of people with diabetes in India currently is 65.1 million, which is expected to rise to 142.7 million by 2035. [16] Mean age of the study population was 51 years, which is in par with the previous studies in India. [17]

We found that mean age was higher in type2 DM with foot ulcer patients than others and that was statistically significant (p<0.0001).Present study found that male had more prevalence in Type2 DM with Foot Ulcer and it was statistically significant (p<0.0001). In type2 DM with foot ulcer, higher number of

patients 16(32.0%) were house wives. In non-diabetic foot ulcer, higher number of patients 28(56.0%) were house wives. In type2 DM without foot ulcer, higher number of patients 26(52.0%) were house wives. In healthy control, higher number of patients 29(58.0%) were house wives. Association of occupation vs. group was not statistically significant (p=0.0002).

Oxidative stress, an imbalance between production of reactive oxygen species (ROS) and cellular antioxidant defence mechanism play an important role in the pathogenesis of Type 2 diabetes and its long-term complications.[18] Chronic psychological stress causes persistent elevation of circulating stress hormones like Adrenaline, Glucagon, Corticosteroids etc. and increased production of Free radicals (ROS - Reactive oxygen species).[19]

Impaired wound healing is a well-documented phenomenon both in experimental and clinical diabetes. Earlier, delayed wound healing was reported together with low collagen content, breaking strength, and increased malondialdehyde levels (an end product of lipid peroxidation due to MPO activity) in diabetic mice, compared to healthy ones. The study suggested that an increased lipid peroxidation in diabetic might have a role in determining a defect of wound repair. Apart from being a potent antimicrobial system, the oxidizing activity of the MPO-H2O2-halide system could elicit inflammatory reactions and tissue injury.[269]Also, antioxidant status is impaired in diabetics compared to normals.[20, 21]With regard of drug metabolism, phenotypes for CYP polymorphism range from ultrarapid to poor metabolizers. In this review, we discuss some of the most clinically important CYPs isoforms (CYP2D6, CYP2A6, CYP2C19, CYP2C9, CYP1B1 and CYP1A2) with respect to gene polymorphisms and drug metabolism. Moreover, the role of Cytochrome p450 Mono Oxygenase in renal, lung, breast and prostate cancers and also discuss their significance for atherosclerosis and type 2 diabetes mellitus.[22]

We found that activation of Cytochrome p450 Mono Oxygenase level was significantly lower in Type2 DM with Foot Ulcer than Healthy Control (t=8.5553). T-test showed that mean Cytochrome p450 Mono Oxygenase of Type2 DM with Foot Ulcer patients was significantly lower than others. Over expression of Neutrophil Myeloperoxidase level was significantly higher in Type2 DM with Foot Ulcer than Healthy Control (t=57.7285). T-test showed that mean Neutrophil Myeloperoxidase of Type2 DM with Foot Ulcer patients was significantly higher than others.

CONCLUSION

It was found increased expression of Cytochrome p450 Mono Oxygenase in types of diabetes mellitus with Foot Ulcer. Adverse Foot Ulcer events associated with Type II diabetes may be in part a result of enhanced Cytochrome p450 Mono Oxygenase expression and activity. Due to this oxidative stress and increased MPO activity, diabetic patients fail to kill the pathogens and heal the wounds in foot ulcer.

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