Dicle Tıp Dergisi
Dicle Medical Journal
2011; 38 (2): 129-133
doi: 10.5798/diclemedj.0921.2011.02.0001

ORIGINAL ARTICLE / ÖZGÜN ARAŞTIRMA

Evaluation of role of Low Molecular Weight Heparin and Vitamin E on renal functions in patients with diabetic nephropathy

Diyabetik nefropatili hastalarda Düşük Molekül Ağırlıklı Heparin ile Vitamin E'nin böbrek fonksiyonları üzerine etkisi

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Geliş Tarihi / Received: 14.03.2011, Kabul Tarihi / Accepted: 29.04.2011

ABSTRACT

Objectives: Diabetes has been reported to increase the risk of end stage renal disease. To prevent the progression of diabetic nephropathy (DN) search for newer approaches is warranted as diabetic patients despite good metabolic control show progressive renal damage. The aim of this study was to investigate the role of low molecular heparin (LWMH) and vitamin E in patients with diabetic nephropathy.

Materials and methods: Twenty five clinically proved adult cases of DN with adequate glycemic and blood pressure control achieved with insulin or oral hypoglycemics and anti hypertensive agents (excluding ACE inhibitors and ARBs) were included. At the start of the study, patients were put on LWMH (3200 IU S/C once daily) for one month and thereafter a washout period of one month was given, following which patients received anti-oxidant vitamin-E (200mg, once daily) for three months.

Results: Patients at the baseline have microalbuminuria levels ranging for 41.0 to 290.0 mg/ 24hr. One month of LMWH reduced it from 133.0±74.4 to 93.3±62.1 mg/24hr ($p<0.001$). Vitamin E supplementation for 3 months did not bring any statistically significant change in the values of microalbuminuria which varied from 95.2±13.0 to 94.2±12.9 mg/24hr ($p>0.05$). The microalbuminuria levels did not revert back to the baseline till the end of the study (5 months).

Conclusion: Our results indicated that, though not a part of standard regime, LMWH treatment followed by vitamin E supplementation needs to be considered in the incipient stage of DN.

Key words: Low molecular weight heparin, vitamin E, diabetic nephropathy, renal functions

ÖZET

Amaç: Diyabetin son dönem böbrek yetmezliği riskini artırdığı bildirilmiştir. Diyabetik nefropatinin ilerlemesini önlemede yeni yaklaşımlar gereklidir, çünkü iyi metabolik kontrole rağmen diyabetik hastalar ilerleyici böbrek hasarı gösterir. Bu çalışmanın amacı düşük molekül ağırlıklı heparin ve vitamin E'nin diyabetik nefropatili hastalardaki rolünü araştırmaktır.

Gereç ve yöntem: Klinik olarak kanıtlanmış, yeterli glisemik kontolu ve kan basıncı kontrolu insulin, oral hipoglisemikler ve kan basıncı düşürücüler (ACE inhibitörü ve ARB'ler hariç) ile sağlanmış, diyabetik nefropatisi olan 25 erişkin hasta çalışmaya dahil edildi. Çalışmanın başlangıcında hastalara DMAH (3200 Ünite, subkutan günde bir kez) bir ay verildi ve takiben bir ay beklenip daha sonra üç ay süreyle E vitamini (Günde bir kez 200 mg) verildi.

Bulgular: Hastaların bazal mikroalbuminüri değerleri, 41.0 ile 290.0 mg/24 st arasında değişiyordu. Bir aylık DMAH bu değeri 133.0 ± 74.4 mg/24 st'den 93.3 ± 62.1 mg/24 st'e düşürdü ($p<0.001$). Üç ay süreli vitamin E suplementasyonu mikroalbuminüri değerinde istatistiksel olarak anlamlı bir değişiklik yapmadı (Sırasıyla, 95.2 ± 13.0 mg/24 st ve 94.2 ± 12.9 mg/24 st, $p>0.05$). Mikroalbuminüri değerleri çalışma süresi olan 5 ayın sonunda başlangıçtaki bazal değerlere dönmemiş olduğu görüldü.

Sonuç: Çalışmamızın sonuçlarına göre, standart tedavi rejiminin bir parçası olmasa bile, DMAH ve takiben vitamin E tedavisinin diabetik nefropati beklenen dönemde dikkate alınması gerekir.

Anahtar kelimeler: Düşük molekül ağırlıklı heparin, vitamin E, diyabetik nefropati, böbrek fonksiyonları

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INTRODUCTION

The development of sustained proteinuria is the major criterion for the diagnosis of diabetic nephropathy (DN). The albuminuria in the range of 30 to 300 mg/day is known as microalbuminuria. Persistent microalbuminuria is considered to indicate high risk for ultimate progression to renal failure.[1]

The search for newer approaches for the prevention and treatment of DN is warranted because even diabetic patients responding to ACEI therapy and metabolic control show progressive renal damage and eventually ESRD.[2] In view of these changes amongst the number of drugs being currently investigated, glycosaminoglycans (GAGs) appeared particularly interesting because they target the generalized endothelial dysfunction and the metabolic defect in the matrix and basement membrane synthesis, which are responsible for DN.[3,4] Heparin, Low molecular weight heparin (LMWH) and other GAGs have been reported to prevent diabetes-induced albuminuria, loss of anionic sites and thickening of the GBM and glomerulosclerosis.[5-14]

Oxidative stress has also been implicated as an important causative factor in the progression of DN.[15,16] Treatment with antioxidants such as Vitamin E may help in ameliorating the effects of oxidative stress in the progression of DN.

This prospective study was planned to evaluate the efficacy of LMWH in patients with DN. A treatment of vitamin E following heparin was chosen to cover the oxidative stress. The study becomes all the more important in the light of recent studies which have shown that a delay in controlling diabetes leads to certain nephropathic changes which may not be easily reversed completely.[17]

MATERIALS AND METHODS

The study was conducted at Pt B.D.Sharma Post Graduate Institute of Medical Sciences, Rohtak. Twenty five clinically proved adult cases of type II diabetes mellitus with diabetic nephropathy (DN) attending medicine OPD were taken up for the study. It was an open and prospective study conducted over a period of five months. All the patients were detailed about the study and pre-informed written consent was obtained before the study. Before subjecting the patients to study adequate glycemic and blood pressure control was achieved with Insulin or Oral hypoglycemic and anti hypertensive agents excluding ACE inhibitors and ARBs. The study period was divided into three parts. At the beginning of study patients were put on low molecular weight heparin {Injection Fluxum (Parnaparin sodium) 3200 IU S/C once daily} for a period of one month. Thereafter a washout period of one month was given, following which patients were put on anti-oxidant therapy (vitamin-E 200 mg) once daily for 3 months.

Patients of diabetes mellitus with stage III diabetic nephropathy were included in the study1. Patients excluded from the study were those with unstable angina, uncontrolled hypertension, heart failure, cirrhosis and those suffering from any other renal disease. Patients with bleeding disorder and malignancy (central bleeding), pregnant or lactating mothers, women of child bearing age, who had taken heparin for any reasons in last 3 months, or were on aspirin and other anti platelet drugs or had hypersensitivity to heparin and related products were also excluded from the study.

All the patients were subjected to investigations like complete haemogram, various renal biochemical parameters, urine complete examination, 24 hr urine for protein and creatinine and creatinine clearance was estimated. Special investigations like microalbuminuria by spectrophotometry, reduced glutathione levels and malonialdehyde levels were also estimated.

All these tests were done at the start (basal), after one month of heparin treatment, after washout period (two months), and following three months of vitamin E therapy.

The data obtained was subjected to standard statistical analysis. For comparison paired samples ‘t’ test was applied. P values were calculated and $p<0.05$ was considered to be statistically significant.

RESULTS

Totally 25 patients enrolled in the present study there were 12 males and 13 females. The age profile of patients showed that a maximum of 40% patients were in the age group of 40-49 which was followed by 32% patients above the age of 50 and the rest of 32% were below 39 and above 20 years. The blood sugar of the patients remained controlled throughout the study.

Patients at baseline had microalbuminuria levels ranging from 41.0 to 290.0 mg/24hr. Microalbuminuria decreased significantly from a baseline value of 133 ± 14.9 to 93.3 ± 12.4 by 1 month of LMWH ($p< 0.001$). Following one month of washout period the levels increased slightly from 93.3 ± 12.4 to 95.2 ± 13.0 but it was not significant statistically ($p>0.05$). But the fall was significant when the wash out period was compared with the base line showing a decrease from 133 ± 14.9 to 95.2 ± 13.02 ($p<0.001$) [Table 1]. Further, Vitamin E supplementation for 3 months did not bring any statistically significant change in the values of microalbuminuria which varied from 95.2 ± 13.0 to 94.2 ± 12.9 ($p>0.05$). However on comparison with the baseline values, the levels continued to remain lower ranging from 133 ± 14.9 to 94.2 ± 12.9 ($p<0.001$) [Table 2]. Thus the earlier effects of LMWH treatment continued to persist up to 4 months (1 month washout + 3 months vitamin E).

Table 1. The effect of low molecular weight heparin on microalbuminuria levels

Parameters	Baseline Mean ± SD I	LMWH treatment (1 month) Mean ± SD II	Washout period (1 month) Mean ± SD III	P value I vs II	P value II vs III	P value III vs I
Microalbuminuria (mg/24hr)	133 ± 14.88	93.3 ± 12.42	95.2 ± 13.02	P<0.001	P>0.05	P<0.001

Table 2. The effect of vitamin E on microalbuminuria levels

Parameters	Baseline value (Mean ± SD) I	Before vitamin E (Mean ± SD) II	After Vitamin E (3 months) (Mean ± SD) III	P value II vs III	P value I vs III
Microalbuminuria (mg/24hr)	133 ± 14.88	95.2 ± 13.02	94.2 ± 12.88	P<0.05	P>0.001

Table 3. The effect of low molecular weight heparin and vitamin E on reduced glutathione and malondialdehyde levels

Parameters	Baseline ± SD	LMWH treatment (1 month)*	Washout period (1 month) *	After 3 months of Vit E**
GSH (mg%)	10 ± 0.09	10.18 ± 0.10	10.27 ± 0.09	13.32 ± 0.18
MDA (µmol/L)	2.41 ± 0.11	2.39 ± 0.18	2.32 ± 0.10	1.69 ± 0.06

*All the values were not significantly different from the baseline values at $p>0.05$
**All the values were significantly different from the baseline values at $p <0.001$

Heparin did not affect GSH levels, which showed a variation from 10.11 ± 0.09 to 10.18 ± 0.10 which was non significant statistically($p>0.05$). Further wash out period also showed no variation ($p>0.05$) Malonialdehyde (MDA) levels also remained unaffected by heparin treatment indicating that LMWH had no antioxidant activity (Table 4).However treatment with vitamin E significantly increased the levels of GSH from 10.27 ± 0.09 to 13.32 ± 0.18 ($p<0.001$), which was in accordance with the antioxidant properties of vitamin E .Similarly malonialdehyde levels also show a highly significant reduction from 2.32 ± 0.10 to 1.69 ± 0.06 ($p<0.01$) following vitamin E, indicating that vitamin E had a role in alleviating oxidative stress in patients of diabetic nephropathy (Table 3).

DISCUSSION

Microalbuminuria is considered as one of the first clinical markers of DN1 and is commonly considered either hemodynamic in origin due to endothelial dysfunction or biochemical, due to alteration in glomerular basement membrane glycosaminoglycan composition, leading to an abnormal permselectivity4. The data presented in Table 1 shows that microalbuminuria decreased significantly by 1 month of LMWH. Our study is in line with Mayrup et al who reported a significant decrease (30-35%) in microalbuminuria/24 hour UAE after treatment with LMW-heparin (2000 anti Xa IU injected subcutaneously) or unfractioned heparin (5000 IU) for 3 months in type 1 diabetic patients. However, their measurements after 3 months could not ascertain

that the progression in albuminuria could be delayed by LMWH treatment or not.[6] In our study on the other hand, it was observed that the microalbuminuria levels continued to remain below the baseline levels till the end of the study. Studies using different GAGs and their combinations have also reported a generalized reduction in microalbuminuria in diabetic patients.[6-14]

The only report from the literature which shows that heparin does not affect microalbuminuria in type 2 diabetic patients used Tinzaparin, a low molecular weight heparin. This was because Tinzaparin had an average molecular weight of 6500 Daltons versus 4500 Daltons for enoxiparin, and moreover has an anti-factor Xa to anti-factor IIa activity ratio of 1.5:2.5 versus 3.3:5.3. Thus, in molar terms, type 2 diabetic patients received 30% less LMWH. Furthermore, differences in molecular weight and anti-X and anti-II activity may be indirect hallmarks of different renoprotective potency of the heparin formulation used.[18]

Based upon epidemiological data, the genetic differences in the enzymes involved in the sulphation of GAG side-chains of heparan sulphate proteoglycans are of crucial importance in the pathogenesis of DN and the recent in-vitro and in-vivo studies have put light on the role of HS-GAG in the pathogenesis of DN.[19] In addition, various experimental and human studies have shown heparin to reduce proteinuria and prevent thickening of glomerular basement membrane glomerular and anionic charge in diabetic nephropathy.[6-14] Several early studies suggested that glomerular epithelial and endothelial cells produce a heparin like substance that inhibits the mesangial cell growth glomerular injury; it is conceivable that the production of this substance is decreased allowing cell proliferation and matrix accumulation as observed in streptozotocin diabetic animals and in the renal ablation model.[20] Exogenous heparin may replace the endogenously synthesized thus lowering microalbuminuria in diabetic patients as observed in the present study.

It was further observed in the present study that vitamin E supplementation 200 mg/day for three months did not cause any statistically significant change in the values of microalbuminuria. Thus the earlier effects of LMWH treatment continued to persist for upto 4 months (1 month washout + 3 months vitamin E). The reduction in microalbuminuria after vitamin E therapy has been reported by Hirnerova et al who after using a very high dose of vitamin E (1200 IU/day for 4 months) observed that microalbuminuria was significantly reduced in the patients of DN after 2 and 4 months of therapy.[21] Yokoyama et al using a lower dose of 600 mg/day vitamin E found Urinary albumin excretion to decrease significantly by 10.8% in patients with good glycemic control (HbA1c < 7.0%).[22] However, Nakamura et al did not find any change in microalbuminuria in type 2 diabetic patients given vitamin E at 300 mg/day.[23] In the present study microalbuminuria levels continued to remain lower than the baseline till the end of the study (5 months), it is difficult to ascertain whether this effect on microalbuminuria levels was a carry forward effect of heparin for up to 5 months or was it the effect of vitamin E supplementation. Elevated extra and intra cellular glucose concentration results in increased formation of reactive oxygen species (ROS) with concomitant increase in intracellular AGE formation. These AGE s especially N-carboxymethyl lysine, pentosidine accumulate in expanded mesangial and nodular lesions of diabetic nephropathy. ROS up regulate transforming factor –beta1, plasminogen activator inhibitor-1, and extra cellular matrix proteins by glomerular mesangial cells, thus leading to mesangial expansion.[24] Vitamin E enhances plasma and red blood cell level of GSH, hence causing significant improvement in markers of oxidative stress resulting in prevention of micro and macro vascular complication of DM. Vitamin E supplementation has been shown to decrease microalbuminuria in patients of DN and also known to ameliorate renal damage; hence the observed maintenance of microalbuminuria after the cessation of LMWH treatment could be due to the role of vitamin E supplementation in delaying the progression of DN. The studies conducted so far on heparin[8] and other GAGs[8-16] do not support the idea that the effect on microalbuminuria could be a carry forward effect of LMWH because they have not observed the persistence of the effects of heparin/GAGs over longer period of time. Vitamin E supplementation, at a low dose of 200 mg /day might have helped in sustaining the effects of LMWH and in maintaining microalbuminuria levels lower than the baseline levels till the study was terminated.

The present study has several limitations, which include limited number of patients and small duration of treatment. Further all the patients were

Indians and there was no control group for comparing placebo verses heparin treatment. Therefore the results of present study cannot be generalized to all diabetic patients.

Although promising, the studies published thus far have generally been too short-term to clarify whether GAG treatment in diabetic patients is capable of curing DN, instead of simply influencing one of its surrogate end points, albuminuria including the present one. As far as known this is a first study of its kind in India where LMWH treatment followed by vitamin E supplementation have been used to study their effect on microalbuminuria in patients of DN. The results from the study indicate that though not a part of standard regime of treatment of DN, LMWH treatment needs to be considered in the early stage of DN.

However further long term multicentric trials are required to determine whether the benefits observed in the present study could be prolonged by long term use of LMWH treatment.

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