Med. J. Cairo Univ., Vol. 62, No. 2, June (Suppl.) : 51-61, 1994.

Microalbuminuria in Sickle Cell Anaemia : A Possible Prognostic Marker : A Dynamic Study

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Abstract

Renal abnormalities almost always present in sickle cell disease are not well appropriately addressed in the literature from Eastern Saudi Arabia. Microalbuminuria has been suggested as a marker of wide spread vascular endothelial dysfunction. The aim of this study was to investigate microalbuminuria as a prognostic criterion in Eastern Saudi Arabia sickle cell patients and to correlate it with pain severity relief in the presence or absence of piracetam or isoxsuprine : 2 proposed course modifying drugs. Accordingly, proven homozygous sickle cell patients were selected from 756 documented patients pool and were randomly assigned to one of the three groups. A group of 25 patients received piracetam therapy, another group of 20 patients received supplement isoxsuprine and the third group of 20 patients received only conventional classic therapy (control group). The study was carried out for 18-24 months. Microalbuminuria was present in 58.3% at the start accounting for 16/25, 12/20 and 10/20 in piracetam, isoxsuprine and control groups respectively. The positive cases of microalbuminuria at the end of the study were 18/25, 12/20 and 12/20 in the same order. Although highly significant improvement of microalbuminuria level was detected in both piracetam and isoxsuprine groups when cases were evaluated case wise, yet neither mean group level nor positive cases showed significant change. Serum complement C3 showed neither change by time nor difference in between groups. Pain severity as assessed by frequency of ingested analgesic tablet/month, frequency of painful crises necessitating medical assistance/month and patient subjective self evaluation of pain degree and duration demonstrated highly significant improvement ($P < 0.001$ and $P = 0.003$). Unfortunately, no statistical correlation could be found between microalbuminuria and the amelioration of pain (frequency, degree and duration). Therefore, the promising prognostic character of the positively confirmed microalbuminuria needs further follow up of the sickle cell patients for at least 5-10 years.

Introduction

PROPER management of sickle cell disease plays an important role in providing the best care for these patients[1]. Much of the improvement in the prognosis was achieved by better management of complications[2]. Vaso-occlusive phenomena, pain attacks and organ damage result in

excessive morbidity and hospital admission with direct impact on the cost of medical care[3]. The combination of high prevalence of the disorder and the limited diagnostic and sometimes therapeutic facilities means that only treatment based on incontestable laboratory and clinical evidence are feasible[4]. No single clinical or laboratory finding proved to be a consistent predictor of prognosis in sickle cell disease [5]. The aim of this study was to investigate and correlate microalbuminuria and pain severity as a prognostic marker in sickle cell anaemia patients with or without the use of two alleged course modifying drugs.

Material and Methods

The study consisted of a randomised controlled prospective follow up. Sixty-five patients with sickle cell anaemia were selected from 756 patients pool, their age ranged from 12 to 42 years (mean 22 ± 6.7). They were 44 males and 21 females. All had homozygous sickle cell anaemia proved by haemoglobin electrophoresis. Exclusion criteria included age below 12 and associated haemoglobinopathy or red cell enzymopathy that are prevalent in the studied population such as thalassaemia or G6PD deficiency. Besides, a complete urine analysis excluded patients with haematuria and frank proteinuria. Patients were randomly assigned to three groups.

Group 1 «20 patients» : They were considered the control group. They received the standard therapy approved in SCECO polyclinics for management of sickle cell patients namely high fluid intake, 10 mg of folic acid daily, diclofenac sodium 50 mg tablet PRN for maintenance therapy to be supplemented during painful crises by 100 mg of ketoprofen dissolved in 1000 ml saline or dextrose 5% IV drip and tiapride hydrochloride 100 mg IV shot «classic therapy».

Group 2 «25 patients» : In addition to the classic therapy, patients received 5 ml of piracetam syrup containing 1000mg of piracetam tid as maintenance therapy and 3 gm of piracetam in the IV drip during the crises.

Group 3 «20 patients» : They received one tablet of isoxsuprine 20 mg every 6 hours orally and extra oral dose during the crises.

Patients were followed up in the out patient clinic every two weeks for : 1) Frequency of self ingested of the standard oral analgesic «diclofenac sodium 50 mg» (number of the consumed tablets). 2) Frequency of painful attacks needing medical assistance. 3) Patients subjective assessment of pain.

Laboratory assessments were carried out by determination of haemoglobin levels, reticulocytic count, serum billirubin concentration creatinine clearance and serum complement C3 in addition to determination of microalbuminuria and bacterial cell count in urine Laboratory assessment was performed at the start «for the previous 3 months» and at the end of the study «18-24 months». Clinical assessment was simultaneously carried out in addition to an intermediate «after 6 months» evaluation.

Techniques :

1 — Microalbuminuria (6813 Urin-Pak Immuno, Ames Division, Miles LTD, England, UK).

Urine was collected and assessed almost immediately. The assay consisted of a semi-automated immuno-turbidimetric method in which human albumin was precipitated as immune complexes in the presence of large excess of high affinity antibodies and polyethylene glycol. Turbidity was then measured photometrically at 340 nm versus the absorbance of a set of calibrators where 20 mg/24 hours or 20 μ g/min. was considered the upper limit of normal reference value[**6**].

2 — Serum complement C3 was assessed by single immunodiffusion Nor-partigen plates (Behring, Houslow) using a modified Mancini technique[**7,8**].

3 — Bacterial viable count was performed by standard calibrated single use plastic loop holding 1 μ l. of undiluted urine spread over the whole surface of CLED agar (Oxoid). The count was then calculated (multiplying by 1000) and a count of 100,000 bacteria/ml. was considered significant[**9**].

Statistical analysis :

Statistical analysis was carried out using SPSS for windows, release 5.0 package[**10**].

Results

Hemoglobin level, reticulocytic count, serum total bilirubin and creatinine clearance did not change allthrough the study. Other results are shown in table 1-4.

Table (1) : Positive Cases of Microalbuminuria in the Groups Studied.

	Piracetam	Isoxsuprine	Control
Total number	N = 25	N = 20	N = 20
At the start :			
With distinct microalbuminuria	18	14	11
Without significant bacteruria	16	12	10
At the end :			
With distinct microalbuminuria	19	13	13
Without significant bacteruria	18	12	12
Improved	17*	15*	2
Deteriorated	3	2	10*

Distinct microalbuminuria = Urinary albumin excretion rate 20-200 μ g/min.
Significant bacteruria > 100,000 CFU/ml.
* Statistically highly significant (Chi square).

Table (1) shows the number of patients with positive microalbuminuria. Only one case turned to frank proteinuria and was subsequently excluded.

Table (2) : Mean Levels of Microalbuminuria in Patients with Distinct Microalbuminuria.

	At the Start			At the End		
	Number	Mean μg/min.	SD	Number	Mean μ g/min.	SD
Piracetam group	16	47.9	50.2	18	46.7	61.2
Isoxsuprine group		50.2	42.6	12	38.8	22.9
Control group	10	43.8	21.8	12	49.6	28.3

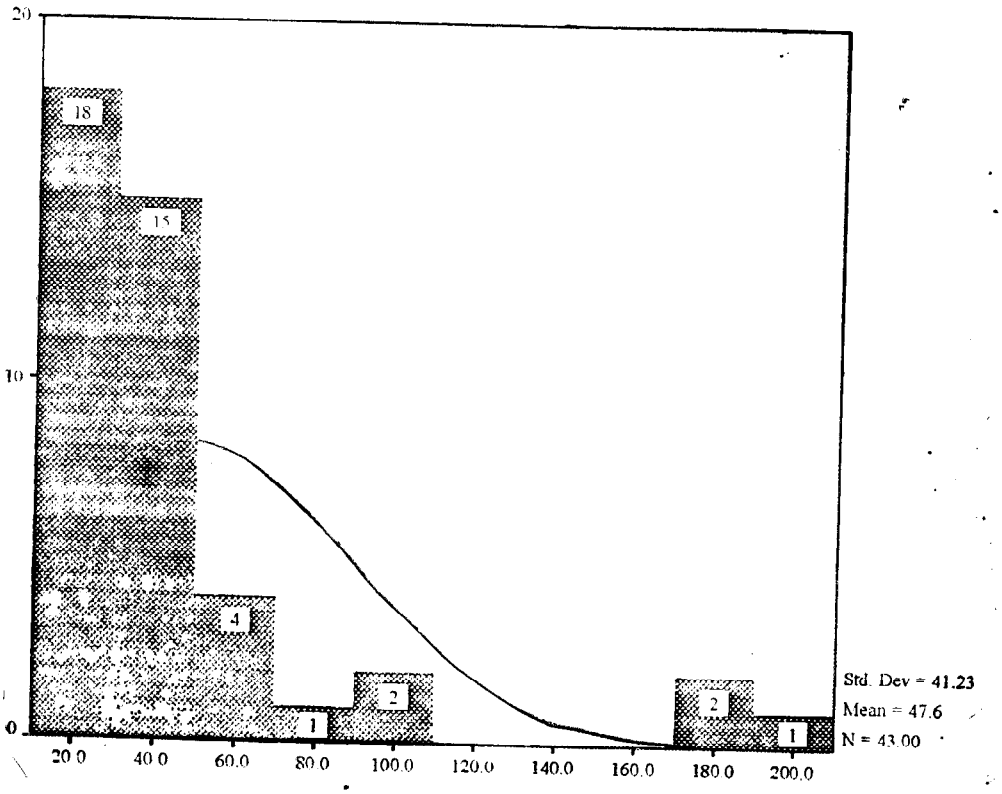

Fig. 1. Distribution of positive microalbuminuria at start (μ g/min.)

Table (3) : Serum Complement C3 Levels in the Three Sickle Patients Groups.

	Piracetam group		Isoxsuprine group		Control group	
	At Start	At End	At Start	At End	At Start	At End
Mean	85.1	79.8	85.8	39.3	84.5	87.7
SD	16.8	17.5	22.3	39.7	26.2	21.3
Patients without Significant bacteruria						
Mean	79.3	77.7	90.9	102.5	90.2	92.1
SD	14.9	18.6	21.7	43.3	28.2	22.2

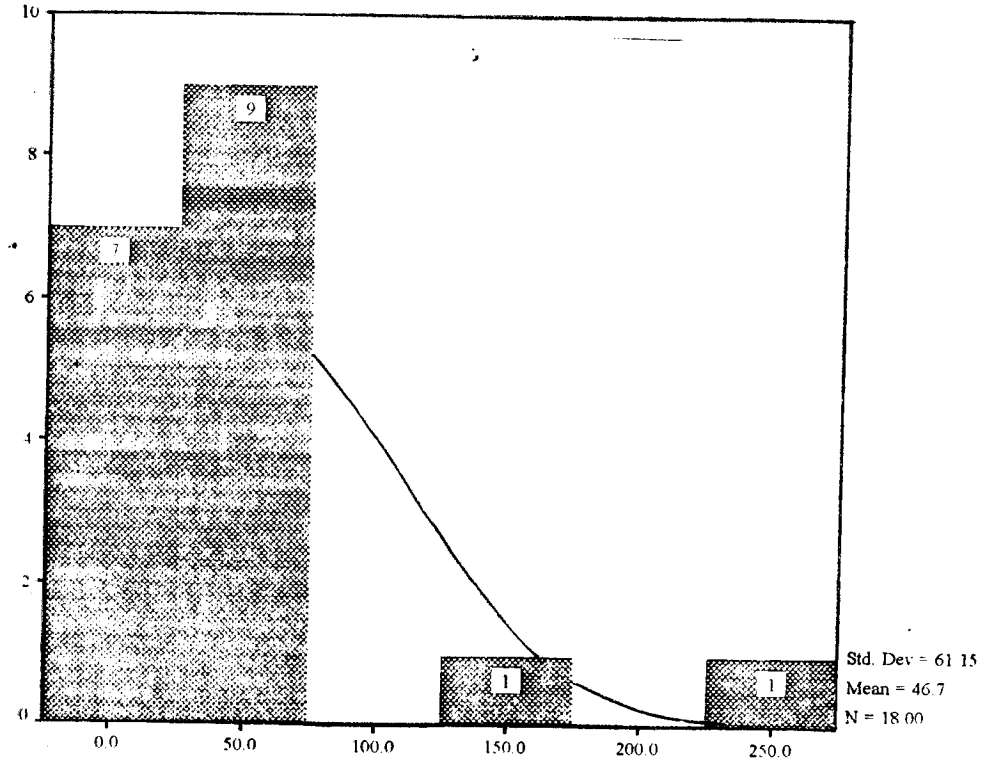

Fig. 2. Distribution of positive microalbuminuria at end in piracetam gp.

As shown, in table 1 both piracetam and isoxsuprine significantly improved the microalbuminuria.

Although a decrease was noted in mean levels of microalbuminuria in drug receiving patients (table 2) more marked with isoxsuprine, the changes were statistically insignificant ($p = 0.67$, $p = 0.55$ and $p = 0.7$ respectively).

Table (4) : Summary of the Clinical Evaluation of Pain in the Three Studied Groups.

		Piracetam group			Isoxsuprine group			Control group		
Time of assessment		0 month	6 months	18-24 months	0 month	6 months	18-24 month	0 months	6 month	18-24 months
Frequency of pain	mean	1.47	1.2*	12.*	1.64	1.6	1.4*	106	106	106
crises/month	SD	0.56	0.32	0.32	0.65	0.6	0.5	0.54	0.54	0.5
Number of analgesic	mean	27	17.4*	16.5*	27.6	20.5*	20.4*	27.4	27.5	27.5
tablets/month	SD	4.2	4.3	3.7	3.8	3	2.3	4.7	4.3	4.7

* Highly significant improvement using single tailed T test for paired samples at 95% CI.

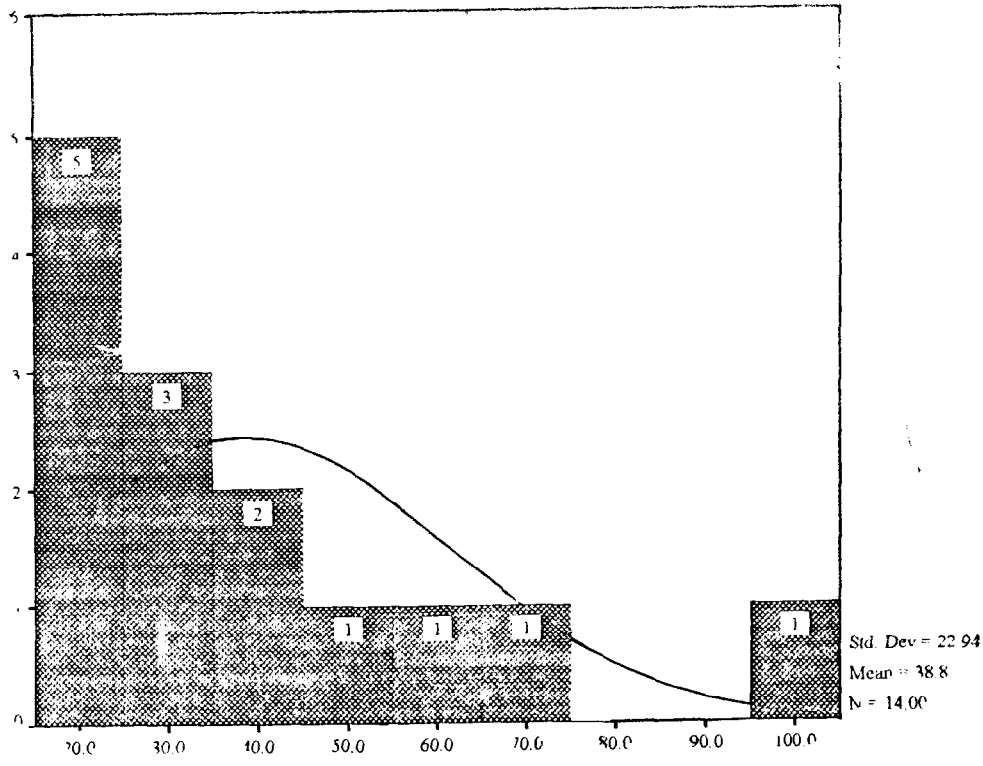

Fig. 3. Distribution of positive microalbuminuria at end in isoxsuprine gp.

The distribution of microalbuminuria is shown in figures 1-4.

The results of assessment of serum complement C3 are shown in table 3. These changes in the level of C3, however, were statistically insignificant.

In addition, all patients in piracetam and isoxsuprine groups reported definite improvement in the degree and the duration of the pain in contrast to five patients only in the control group.

Discussion

Painful vaso-occlusive crises are the primary cause of morbidity of sickle cell anaemia, even in its benign form prevalent in Eastern Saudi Arabia. In addition to pain, almost every organ may be damaged, most commonly lungs, kidneys, liver, skeleton and skin[5]. Microalbuminuria has been suggested as a marker of wide spread endothelial dysfunction[11]. In this study, we tried to study microalbuminuria and pain with or without the use of two drugs supposed to modify the course of the disease.

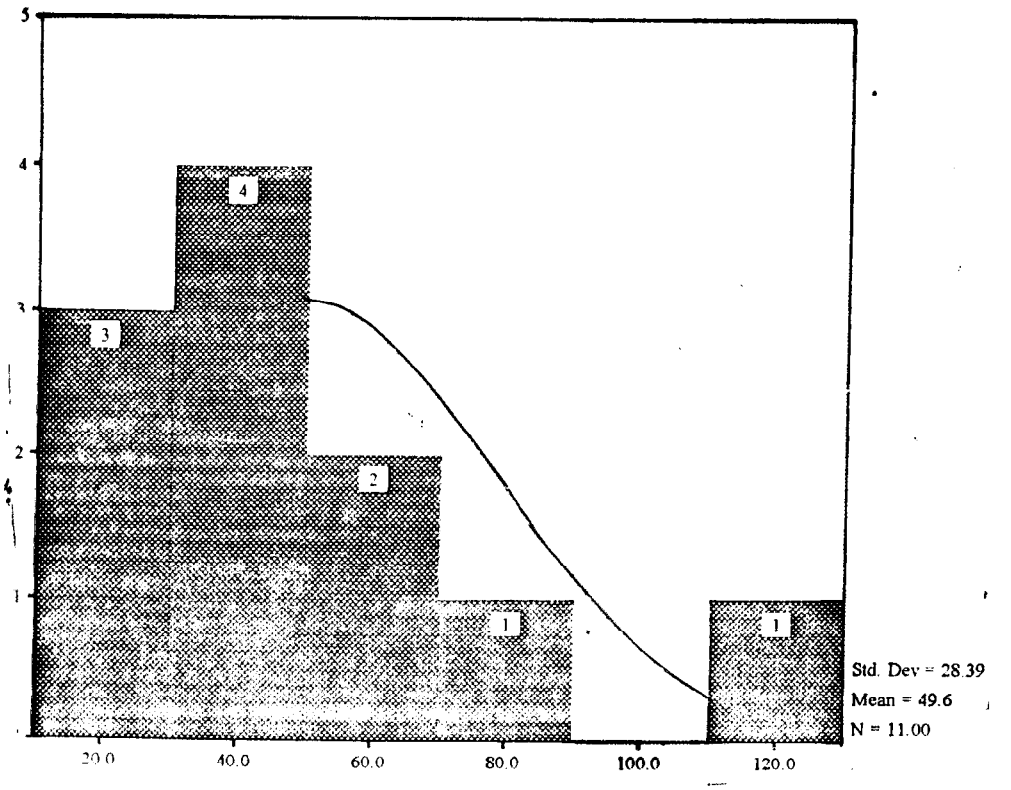

Fig. 4. Distribution of positive microalbuminuria at end in control gp.

The number of patients with improved microalbuminuria, at the end of the study, were 17/25 in the piracetam group ($P < 0.005$), 15/20 in the isoxsuprine group ($P < 0.005$) and 2/20 in the control group. In fact the control group rather showed significant deterioration «10/20, $P < 0.01$». Although the mean level of microalbuminuria in the piracetam and isoxsuprine groups were less than that of the control group at the end of the study, yet the differences were statistically insignificant even if corrected for only the improved or deteriorated patients. To our knowledge, no published data addressed the subject of microalbuminuria in Eastern Saudi Arabia sicklers. Renal dysfunction is nearly always present in patients with sickle cell anaemia[**12**]. Unfortunately this problem was not addressed properly in the available literature[**13**]. Glomerular sclerosis is an established direct consequence of sickle cell disease[**14**]. Beside the possibility of haemodynamic glomerular injury precipitated by a state of renal hyper perfusion, glomerular hypertrophy and hyper-filtration, an immune complex mediated glomerulonephritis has been postulated as immune complexes were detected in sickle cell patients[**15,16**]. Proteinuria is found in 29 to 50% of adult patients with sickle cell anaemia[**3**]. The

prognosis of large number of glomerular condition was proved to be related to the presence and/or the magnitude of proteinuria[17] including membranous nephropathy[18], focal segmental glomerulosclerosis[19], mesangiocapillary glomerulonephritis[20], diabetic nephropathy[21], reflux nephropathy[22/, acute endocapillary glomerulonephritis[23] and IgA associated nephropathy[24]. So the improvement of microalbuminuria in the piracetam and isoxsuprine group might denote regression or at least stabilization of the glomerular lesion in these patients. In contrast in the control group the deterioration of microalbuminuria may denote substantial progression of the glomerular lesion. These minor changes did not affect the creatinine clearance which showed no significant changes patient wise as well as group wise. The results of this study do not support nor deny the immune complex mediated glomerular injury as there were no significant dynamic changes in serum complement C3 levels group wise or in between the three groups. In another series of adult patients from the same original population the mean serum complement C3 level was found to be closely similar 78.8 mg/dl (n = 26) while the mean level of the corresponding non-sicklers assayed simultaneously was 84.3 mg/dl (n = 30) with no statistically significant difference[25].

The results of follow up of pain in patients on piracetam revealed a highly significant decrease of pain crises from a mean of 1.47 attacks/month to 1.2 attacks/month after 6 months ($P < 0.001$), while patient on isoxsuprine showed a highly significant decrease of pain crises only at the end of study ($P < 0.005$), with no changes in the control group. Moreover, the number of home self ingested analgesic tablets (diclofenac sodium 50 mg) per month decreased significantly in patients on piracetam and isoxsuprine when evaluated at the 6th. month of study ($P < 0.005$). These patients also reported an improvement by their own subjective assessment of the degree and duration of pain.

Piracetam effect on pain is consistent with the previous results in acute vaso-occlusive crises[26] and in the prophylaxis of such crises[27,28]. The action of piracetam seems to be on multiple target. It is capable of improving the microcirculation by suppressing platelet activity in vivo[29,30], restores the deformability of Hb SS erythrocytes through reduction of incorporation of phosphorous into specterin related membrane proteins, an activity proven by in vitro filtration of sickle erythrocytes through a neucleoporous membrane with pores of 5 microns [31]. Piracetam also re-establishes the normal hydration of sickle cells and corrects the excess of adhesion of these cells to the vascular endothelium. This excessive adhesion is thought to be one of the initiating factor of a circulatory slow down resulting in a peripheral sickling due to anoxia and a state of vaso occlusion[28].

Psomodakis et al were the first to report that isoxsuprine exerts a remarkable action in sickle cell crises by bringing prompt relieve of pain, reduction of narcotic administration and shortening the hospitalization time[32]. They explained

the action of isoxsuprine by local vaso dilatation which eventually breaks the vicious circle of sickling, hypoxia and vasospasm. Isoxsuprine by increasing the cardiac output results in a decrease of the arterio-venous oxygen difference which maintain higher venous oxygen level as well as shortening the circulation time with subsequent reduction of red cell transient time in area of low oxygen tension[**33**].

Conclusion :

Piracetam and isoxsuprine are effective in amelioration of sickle cell disease pain through significant reduction of vaso occlusive crises requiring medical assistance associated with reduction of self ingested analgesic tablets as well as marked improvement of microalbuminuria level. Microalbuminuria was detected in 38/65 i.e. 53.3% of the selected non proteinuric patients, and as almost 50% of adult sickle cell patients turn to be proteinuric, those patients with microalbuminuria might have early nephropathy that would eventually progress to frank nephropathy and proteinuria. Thus microalbuminuria might show to be a predictor marker for sickle cell nephropathy. Moreover, the improvement of microalbuminuria levels after the use of piracetam and isoxsuprine with clinical improvement of pain tends to strengthen a second tempting postulate that microalbuminuria might be a prognostic marker for all vascular complications of sickle cell anaemia. Unfortunately, we could not find statistically significant correlation between these dynamic changes. We recommend to follow up these patients for further 5 to 10 years to achieve solid confirmation for these two postulates

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