RESEARCH

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Microalbuminuria as a predictor of early glomerular injury in children and adolescents with Sickle Cell Anaemia at King Salman Armed Forced Hospital, Tabuk, Saudi Arabia

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Introduction: **Microalbuminuria (MA) is considered as an early marker of various diseases affecting the renal system. Its relevance in children with sickle cell anaemia (SCA), who are known to be prone to renal complications, has not been fully explored. Microalbuminuria in the early stages of sickle cell nephropathy is a hallmark of future deterioration of renal function.** ***Objective:*** **The objective of this study was to determine the prevalence of microalbuminuria and its clinical correlates in children and adolescents with sickle cell disease attending sickle cell anaemia clinic at King Salman Armed Forced Hospital (KSAFH).** ***Methods:*** **This is a hospital based case-control study that was conducted in KSAFH in the period from 2014-2016. A total of 145 patients aged 3 – 18 years attending sickle cell clinic were randomly selected. These children were divided into 3 groups. Group A Sickle cell anaemia (69 patients), Group B sickle thalassemia (13 patients), Group C sickle cell trait (10 patients) along with 54 healthy children of comparable age as controls. The demographic data including age, sex, and residency were analysed. A urine sample of all eligible children of the 3 groups together with controls were collected and analysed and screened for microalbuminuria. The haemoglobin level with microalbuminuria was recorded and compared between the 3 groups and the controls.** ***Results:*** **The mean age of patients was found to be 8.5 years and 44.6% were females. Microalbuminuria (MA) was found in 42/145 (28.9%) and it was much more common in males than in females 61.9% and it was more common at a higher age. None of the clinical characteristics (painful crisis, blood transfusion) was significantly related to MA. Haemoglobin levels were significantly lower in subjects with MA than in those without MA.** ***Conclusions:*** **Microalbuminuria was a insignificant number of patients with sickle cell disease and was more common in males and higher age. In conclusion, the prevalence of MA in children (1-16 years) with SCA in our study was 28.9%. Prevalence of MA was influenced by age, gender.**

INTRODUCTION

The sickle cell nephropathy (SCN) encompasses all the structural and functional abnormalities of the kidneys seen in Sickle cell disease (SCD)[1]. SCN manifests with various glomerular and tubular abnormalities such as proteinuria with/without nephritic or nephrotic syndrome[2]. Patients with SCA who have impaired renal function could benefit from enhanced care through interventional measures aimed at minimizing progression of proteinuria, such as with the use of angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, aldosterone, etc[3]. Dipstick urinalysis cannot detect proteinuria below the value of 300 mg/dL[4], defined as microalbuminuria (MA) that heralds early renal involvement. In recent years, therefore, interest has shifted to testing for MA in a variety of clinical diseases such as diabetes mellitus[5, 18], hypertension[6, 19, 20] and sickle cell disease (SCD)[7, 8]. MA is the excretion in urine of very small amounts of albumin, slightly in excess of 20 µg/min[9], in the range of 30-300 mg/24 hours[10], levels that require sensitive radioimmunoassay for detection[7]. MA has also been found to be an important marker of glomerular injury in patients with sickle cell anaemia (SCA)[8]. MA detection using the Micral-test strip (commercial test strips that detect MA in spot urine) has now become useful in this regard. These manifestations are results of chronic renal microvascular occlusion by sickled erythrocytes, the effect of which is accentuated

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during crises[11, 12, 13]. Although MA has been found to be useful in early detection of renal impairment, and hence, the early detection of SCN as well[14]. There was scanty of research in the Middle East region especially in Saudi Arabia, therefore, this study was designed to explore the presence of MA in children with SCA, to evaluate as a potential marker of early renal impairment.

METHODOLOGY

This study is prospective and cross-sectional that was carried out at the king Salman North Armed Forced Hospital in the haematology clinic in Tabuk city, Saudi Arabia. The period of study was from November 2014 to February 2016. The study recruits a total of 145 subjects further classified into 4 groups.

Group 1: Sickle cell anaemia,
Group 2: Sickle thalassemia,
Group 3: Sickle cell trait,
Group 4: Control subject.

Inclusion criteria:
A confirmed diagnosis of SCA by Hb Electrophoresis. Age range from 3 to 18 years at the time of enrollment. Written consent was taken from all parents and guardians of all eligible children and adolescent.

Exclusion Criteria:
Children who had a co-existing renal disease such as nephrotic syndrome, Clinical signs of acute infection at the time of the investigation.

Clinical:
Albuminuria, was defined when the urine albumin is above 20mg/L or 0.02g/L. Microalbuminuria defined as Albumin to creatinine ratio(ACR) between (30- 300)mg/g. A thorough physical examination was carried out on each child, checking for pallor (anaemia), jaundice, peripheral edema, leg ulcers, and organomegaly. Findings of the study on the subjects were recorded in the study files designed for the purpose.

This study utilized existing and prospectively collected data from the ongoing SCD patients. The database of the clinical study was used to ascertain information regarding the SCD status Structured questionnaires were used to collect information from the children and adolescents, including social demographic data, clinical events, and laboratory results of complete blood count. All enrolled subjects were provided with the pre-labelled universal bottle for the collection of the urine. The urine sample was first tested for Macroalbuminuria (Proteinuria) using the normal Urodip-10 strips (Germany). This test cannot detect protein level in urine below the value of 300mg/L. The samples that were negative for Proteinuria was subsequently tested for MA using Microalbumin 21 test strips (Cliawaived Microalbumin 2-1 Combo, USA), capable of detecting as low as 20mg/L. About 2 mL of venous blood was collected from each patient by venipuncture (using aseptic precautions) during the second visit, into ethylenediaminetetraacetic acid (EDTA) bottle to confirm the patient's haemoglobin genotype.

The following clinical events were recorded over a period of 12 months. These were sickle cell related clinical events that necessitated admission. Painful crisis, dactylitis, blood transfusion, convulsions, neurological deficit, acute chest illness, priapism, avascular necrosis and aplastic crisis. Episodes of clinical events were defined by the diagnosis recorded in the medical record.

Statistical methodology:
Statistical presentation and analysis of the present study were conducted with SPSS V.20. Data was expressed into two phases: For descriptive data, the mean± standard deviation was used for quantitative analysis and for quantitative data, frequency and percentage were used. Mann Whitney test was used to compare two independent quantitative variables (non-normally distributed). Chi^2 is used to compare two independent qualitative variables (normally distributed). P value < 0.05 was considered statistically significant.

Ethical Clearance:
For every child participant, written informed consent was obtained from parents or legal guardians on behalf of the child. This consent form and the study were reviewed and approved by the Institutional Ethical Committee of the Faculty of Medicine, University of Tabuk, in compliance with the principles of the Helsinki Declaration II. Pseudonyms are used for the children to ensure confidentiality.

RESULTS

A total of 145 study subjects were enrolled, out of which group 1 (sickle cell anaemia) comprises of 46.9% patients. Whereas 9% and 6.9% were recruited in Group 2 and group 3 respectively (table 1). Their mean age is 8.5 years, 38.8% were females. Microalbuminuria (MA) was found in 28.9% subjects and it was much more common in males (61.9%) than in females (38.1%) and is one of the most common at higher age (table 2). Table 3 shows the clinical characteristics of the study population for the determinants of microalbuminuria. In the analysis, None of the clinical characteristics (painful crisis, blood transfusion) significantly related to MA (figure 1). The mean age (9.2±2.9) was higher in group 1 children followed by group 3, group 2 and group 4. There was a significant ant difference in the rate of blood transfusion in the last 2 years (<0.001) (figure 2). The recurrent painful crisis was observed among group 1 and group 2 subjects.

Table 1 The number and % of different study groups in relation to control

Study Groups		N(145)	%
Group 1	Sickle cell anaemia	68	46.9
Group 2	Sickle cell thalassemia	13	9
Group 3	Sickle cell trait	10	6.9
Group 4	Control	54	37.2

Table 2 Age and gender distribution of children with and without microalbuminuria

	Microalbumin			
	<0.03 N=103	>0.03 N=42		P value
Female Male	40(38.8) 63(61.2)	16(38.1) 26(61.9)	0.007**	0.934
Age	8.2±3.3	9±2.6	1.4*	0.174

*U test, **X2

Table 4 shows the biochemical characteristics of the study groups. There was a significant difference among all groups in the mean level of microalbumin (p=0.027). Haemoglobin levels were significantly lower in subjects with MA than in those without MA (5.9±1.2 vs 7.4±1.0g/dL, respectively) p=0.001. In the multivariate logistic regression model of MA, both Hb level and age remain in the final model as clinical correlates of MA. Higher Hb level showed a protective effect against MA (Odds ratio=0.55) p=0.001 while subjects with MA were more likely to have older age. (Odds ratio=1.7) p=0.001. a None of the other parameters (pH, specific gravity, urea and creatinine) shows a

Table 3 Demographic and clinical characteristics of the study population

Gender	**Group 1:** Sickle cell Anaemia N %	**Group 2:** Sickle cell Thalassemia	**Group 3:** Sickle cell trait	**Group 4:** Control	X2	P value
Female Male	28(41.2%) 40(58.8)	0(0%) 13(100%)	5(50) 5(50)	23(42.6) 31(57.4)	9.3	0.026
Family members with HBSS/HbAS One Two Three More Non	 14(20.6) 17(25) 11(16.2) 21(30.9) 5(7.4)	 6(46.2) 3(23.1) 0 4(30.8) 0	 4(40) 2(20) 4(40) 0 0	 2(3.7) 4(7.4) 0 5(9.3) 43(79.6)	104.46	<0.001
Age	9.2±2.9	7.8±2.3	8.9±4.3	8.4±3.4	0.285*	0.836
Blood transfusion in the last 2 years +ve -ve	 33(48.5) 35(51.5)	 13(100) 0	 0 10(100)	 0 54(100)	66.6	<0.001
ICU admission No Yes	 52(76.5) 16(23.5)	 13(100) 0(0)	 10(100) 0	 54(100) 0(0)	20.4	<0.001
Recurrent painful crisis No Yes	 24(35.3) 44(64.7)	 7(53.8) 6(46.2)	 10(100) 0	 54(100) 0	62	<0.001
Polyuria /oliguria No Yes	 61(89.7) 7(10.3)	 7(53.8) 6(46.2)	 10(100) 0	 53(98.1) 1(1.9)	24.7	<0.001
Hematuria No Yes	 60(88.2) 8(11.8)	 13(100) 0(0)	 10(100) 0	 54(100) 0(0)	9.6	0.02
Dysuria No Yes	 54(79.4) 14(20.6)	 13(100) 0(0)	 10(100) 0	 53(98.1) 1(1.9)53	14.5	0.002
A headache No Yes	 55(80.9) 13(19.1)	 13(100) 0(0)	 10(100) 0	 54(100) 0(0)	16.2	0.001
Leucocyte -ve +ve	 64(94.1) 4(5.9)	 13(100) 0(0)	 10(100) 0	 53(98.1) 1(1.9)53	2.4	0.485
Blood -ve +ve	 66(97.1) 2(2.9)	 13(100) 0(0)	 10(100) 0(0)	 51(94.4) 3(5.6)	1.6	0.661
Protein -ve +ve	 67(98.5) 1(1.5)	 13(100) 0(0)	 10(100) 0	 53(98.1) 1(1.9)53	0.414	0.937
Nitrate -ve +ve	 66(97.1) 2(2.9)	 13(100) 0(0)	 10(100) 0	 54(100) 0(0)	2.3	0.513
Ketone -ve	68(100)	13(100)	10(100)	54(100)	---	---
Bilirubin -ve +ve	 68(100) 0	 13(100) 0	 6(60) 4(40)	 54(100) 0	55.5	<0.001
Glucose -ve	 68(100)	 13(100)	 10(100)	 54(100)	---	--

*k test (Kruskal Wallis)

Table 4 Biochemical analysis

	Group 1: Sickle cell Anaemia N %	**Group 2:** Sickle cell Thalassemia	**Group 3:** Sickle cell trait	**Group 4:** Control	K test	P value
pH	6.2±0.7	6.23±0.4	6±0.001	6.06±0.6	0.9	0.443
Specific gravity	1031.1±122.5	1015.4±5.2	1117±313.8	1016.6±6.5	2.2	0.09
Urea	2.9±0.9	2.3±0.6	3.1±0.6	2.7±0.8	2.6	0.052
Creatine	27.3±9.1	27.7±8.2	23.2±4.1	24.7±4.9	1.8	0.14
Microalbumin	0.05±0.01	0.03±0.005	0.01±0.01	0.009±0.003	3.2	0.027

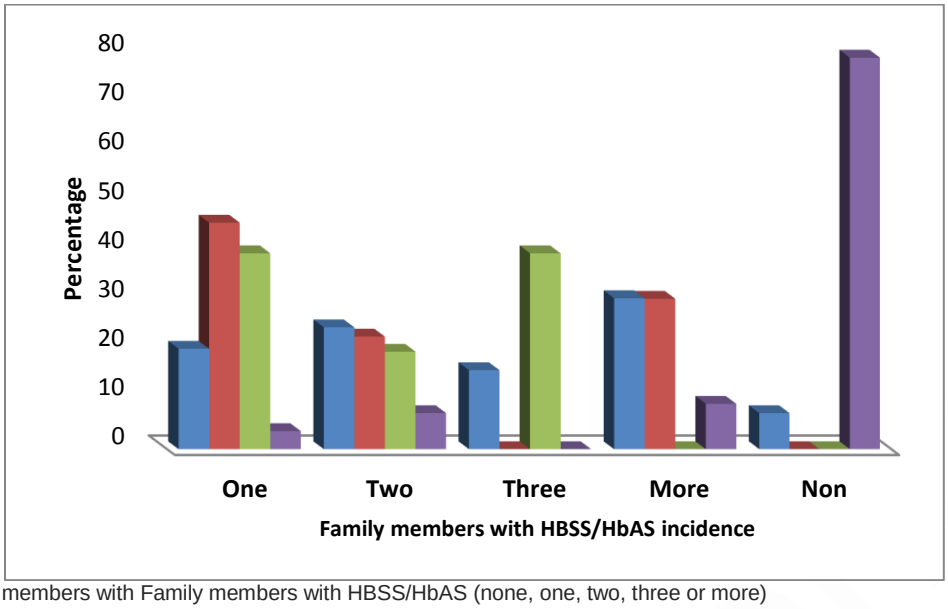

Figure 1 History of family members with Family members with HBSS/HbAS (none, one, two, three or more)

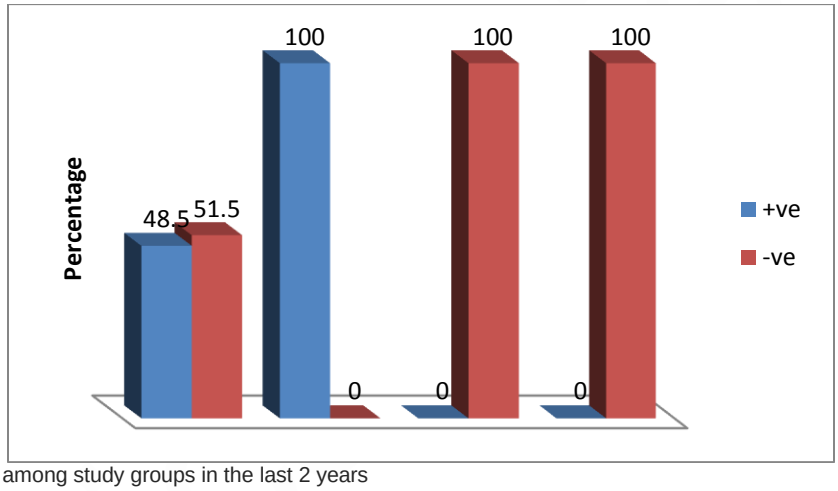

Figure 2 Blood transfusion rate among study groups in the last 2 years

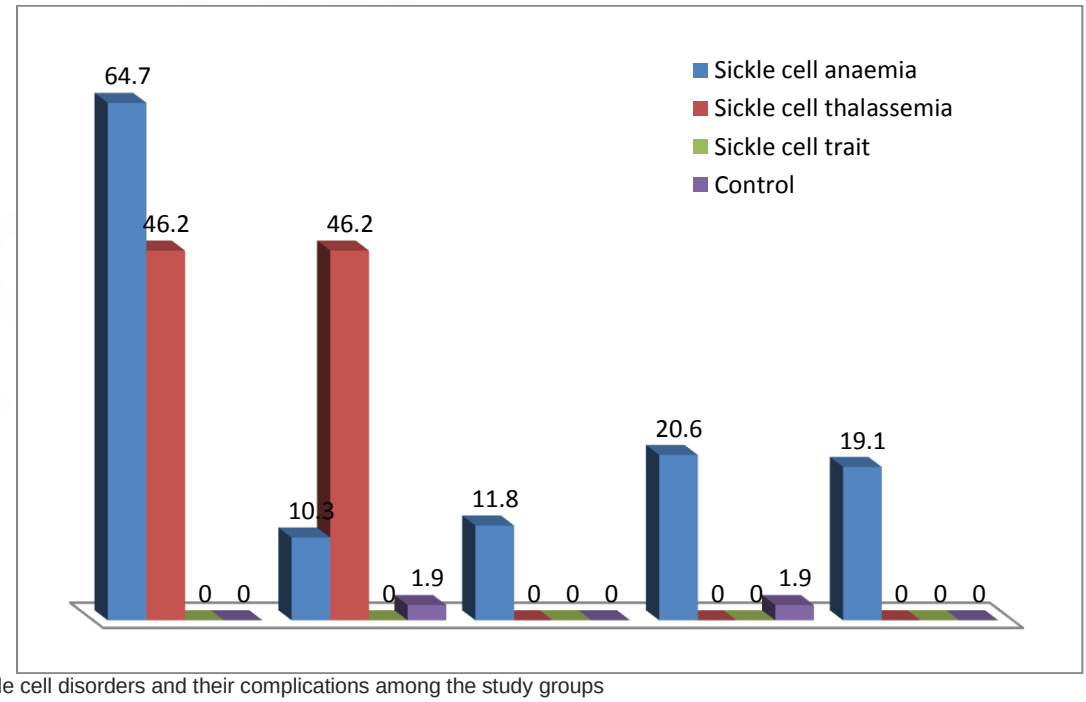

Figure 3 The sickle cell disorders and their complications among the study groups

significant difference among groups (group 1 to 4) (figure 3). However, a statistically significant difference was not observed. Other variables considered did not significantly differ between the groups.

DISCUSSION

In this study, the overall prevalence of MA in children with HbSS was 28.9%. Prevalence figures were low among pre-school aged children and rose in those aged ≥9 years. In consonance with the findings in this study, Datta et al[15] in 2003 reported a 19.2% prevalence of MA among Indian children with SCD. Similarly, Alvarex et al[16] in 2008 recorded a prevalence of 16.8% amongst HbSS children and 18.0% amongst the SC group. They worked on 120 children and young adults between the ages of 4 and 20 years. In the United States, in 2002 found the prevalence of MA in 142 children with SCD to be 19.0%[2]. In yet another American study in 1998 reported on 102 children aged 2-18 years with SCA [1]. They found a high prevalence of 26.5% in their study subjects. However, in children aged 10-18 years, the prevalence was 46%. The difference between their overall prevalence and that found in this study is most likely due to the increased number of older subjects (between 17 and 18 years) recruited for their study compared to our study, as the prevalence of MA increases with increasing age. Datta *et al*[15] in 2003 reported a 19.2% prevalence of MA among Indian children. However, in their study, they included other forms of SCD. Similarly, in the year 2008 recorded a prevalence of 15.8%, the low prevalence could be explained by a higher haemoglobin level of their study subjects[16]. Like what was found by other studies[1,2,12] where MA was not seen in any child less than seven years, this is consistent with our finding. This could be explained by the fact that in their study they only involved subjects with the only HbSS like in the current study. Furthermore in 1998, recorded a prevalence of 30% in Brazil [9], both children and adults with SCA were their study group. The higher figures may, therefore, be ascribed to the fact that older subjects were more recruited for study than younger subjects. In another study, that MA was related to age, with positive linear correlation. The prevalence figures were low among children 8% and rose to 37% in those adolescents[17]. It was also found that a number of admissions, pain episodes, blood transfusion was not associated with the microalbuminuria occurrence to any significant degree[1,2,11,13] , this sounded unexpected since it was thought the more the clinical events could be associated with MA. Our finding also reported similar results.

However, episodes of acute chest syndrome (ACS) were found to be significantly related to MA[16]. This could be explained with another genotype of Hb, but this still remains to be debatable because other studies had another genotype still no association was seen on clinical events. In this study none of our subjects received the treatment to reduce microalbuminuria in urine, others studies[2,11,13,16] have shown treatment with an ACEI or Hydroxyurea has a potentially renoprotective effect in the setting of MA. This could probably explain the lower prevalence of MA in their studies compared to ours.

Study limitation

The study was a cross-sectional design which does not determine the full significance of pediatric MA in the development of kidney disease. The only way to determine the true predictive value of childhood MA is to follow up all subjects into adulthood in a cohort study. This was a hospital-based study hence prone to selection bias. However, since all sickle cell patients in KSAFH were referred to this clinic it reduced the chances of high selection bias. The lowest statistically acceptable sample size was used due to high investigation cost.

CONCLUSION

In this study, it was found that the MA is prevalent in 28.7 % in our setting where the burden of SCA is high. The occurrence of MA is directly proportional to age and inversely proportional to the Haemoglobin level. No association was found between MA and clinical events. Gender had an effect on MA occurrence.

Recommendation

Measurement of urinary microalbumin is simple and non-invasive screening biomarkers, which may be utilized as part of routine health care maintenance in children with SCA. Screening for microalbuminuria seems prudent after age six to seven years of age. Children with lower haemoglobin levels should be monitored closely because they appear to be at increased risk. Routine screening of microalbuminuria will likely be as helpful a predictor of end-renal damage particularly in those subjects with severe anaemia. Longitudinal studies are essential to determine the significance of childhood microalbuminuria in the development of renal disease and then to devise a strategy to prevent sickle cell nephropathy.

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