SIDEROBLASTIC ANAEMIA–A HITHERTO UNRECOGNIZED CAUSE OF UNEXPLAINED ANAEMIA

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ABSTRACT

Objective: To assess the clinicopathological heterogeneity of sideroblastic anemia disorders characterized by the presence of ring sideroblasts in the bone marrow.

Study Design: Descriptive study.

Place and Duration of Study: Study was conducted at department of Pathology, Combined Military Hospital Kharian, from Apr 2014 to Oct 2016.

Material and Methods: A total of ten patients diagnosed as having sideroblastic anemia (SA) on cytomorphological basis were included in the study. After clinical examination of the patients, blood samples were analyzed on Sysmex KX21 Haematology analyzer. Blood and bone marrow aspiration slides were stained with leishman's stain to study red cell morphology and aspects of haematopoiesis. Assessment of iron stores was done using Perl's staining technique. Data was collected and analyzed using SPSS 16.0 version.

Results: Age range of the SA patients varied from 14-65 years and male to female ratio was 1.3:1. Clinical features included weakness, malaise, easy fatigability, fever, bleeding complications, pallor, splenomegaly and syndrome specific features in 2 patients. MCV ranged from 66 fl to 94 fl. Dimorphic red cell morphology which is considered an important feature of SA was not observed in any patient. Other cytopenias were also noticed. Dysplasia was observed in 4 patients. One patient was confirmed as having secondary SA due to lead poisoning.

Conclusion: Clinicopathological features of SA are variable and it is inappropriate to associate SA with any specific age group, gender, red cell indices or morphology. Prompt recognition of SA with its accurate categorization and specific treatment can avoid undue suffering of the patients as well as their relatives.

Keywords: MELAS, Myelodysplastic syndrome, Sideroblastic anemia, Wolfram syndrome.

INTRODUCTION

In erythroblasts, iron is mainly used for haem synthesis. The heme biosynthetic pathway is restricted to the mitochondria. The first step is carried out by the erythroid-specific enzyme delta-aminolevulinic acid synthase 2 (ALAS2). This enzyme is under the control of two iron regulatory proteins (IRP1 and IRP2) acting on the ALAS2 iron responsive element (IRE), which is not present in non-erythroid ALAS[1]. The last step of haem synthesis is the incorporation of Fe2+ into protoporphyrin IX, catalysed by ferrochelatase. Defects involving incorporation of iron into the heme molecule of hemoglobin lead to granular deposition of iron in the mitochondria that form a ring around the nucleus of the developing red blood cells and this results in SA. Disturbed intracellular iron homeostasis including iron sulfur (Fe-S) cluster biogenesis and mitochondrial metabolism defects have also been implicated in the pathogenesis of SA[1]. Because of the diversity of this disorder, variations and imprecision have existed in classifying the disorder. However causes of SA can be categorized into four groups: Congenital syndromic SA, congenital non syndromic SA, acquired reversible SA and acquired clonal SA[2]. The syndromic forms of congenital SA are usually part of multisystem mitochondrial dysfunction disorders. The clinical presentation of mitochondrial disorders can be highly suggestive of a particular disease phenotype.

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There may be well recognised clinical symptoms suggesting specific mtDNA defect and these include pearson marrow pancreas syndrome, Wolfram's syndrome, MELAS (Myoencephalopathy, lactic acidosis and stroke like episodes), Kearn-Sayre syndrome and MLASA (Myopathy, lactic acidosis and sideroblastic anemia)[3], of the congenital non syndromic SA, X linked, autosomal dominant and autosomal recessive forms of inheritance have been described along with clinical and pathological features including genetic abnormalities like ALAS2, SLC25A38, GLRX5, ABCB 7, PUS1, SLC19A2 and HSPA9 gene defects[4,5]. There are still significant number of cases of inherited SA with undefined genetics. Molecular analysis of these cases will contribute not only to the development of effective treatment, but also to the understanding of mitochondrial iron metabolism[6]. The acquired SA has been categorized into reversible and clonal types. Reversible SA is due to toxins like lead and zinc, drugs like chloramphenicol, isoniazid and alcohol or due to certain deficiency states like pyridoxine and copper. Clonal SA is related to various MDS subcategories including refractory anemia with ring sideroblasts (RARS), refractory cytopenias with multilineage dysplasia with Ring sideroblasts (RCMD-RS) and refractory anemia with ring sideroblasts and thrombocytosis (RARS-T). Acquired clonal SA is supposed to be more common than the reversible type and its associated genetic abnormalities like the splicing factor 3B subunit 1 (SF3B1) gene mutation in RARS and the association of JAK2 mutation with RARS-T have begun to be highlighted by researchers[7]. Additional genetic lesions may be involved in the latter two variants and even though the underlying molecular defects are different than those identified in the congenital SA, the theme of effects at the end seems to be common for the congenital and acquired clonal SA categories[8]. There are a number of misconceptions about SA. Among these is its association with a specific age, gender, MCV range and red cell morphology as well as requirement of a particular percentage of ring sideroblasts for diagnosis. The fact that SA is a heterogenous disorder with a multitude of causes itself indicates that its presentation may be as variable as the underlying causes. Here, we present data of ten cases diagnosed as SA over two and a half years at a tertiary care centre and analyze the variation in clinical data and laboratory results to ascertain correctness of the misconceptions about SA.

MATERIAL AND METHODS

This is a descriptive study carried out on ten patients diagnosed as having sideroblastic anemia (SA) on cytomorphological basis at the pathology department of combined military hospital kharian from Apr 2014 to Oct 2016. Patients were selected through non-probability convenience sampling. After relevant history taking and physical examination of the patients, blood samples were taken and subjected to analysis on Sysmex KX21 Haematology analyzer. Peripheral blood smears were prepared and stained with Leishman's stain to assess red cell morphology. Bone marrow aspiration was performed under local anesthesia with aseptic technique. Air dried smears were then stained with leishman's stain to assess cellularity and other aspects of haematopoiesis. Assessment of iron stores was done using Perl's staining technique. Except for acquired clonal SA cases where a 'cut off' of 15% was used, only frequent observation of ring sideroblasts fulfilling the diagnostic criterion of a ring sideroblast was taken as positive finding for SA diagnosis. Data was collected and analyzed using SPSS 16.0 version. Mean and standard deviation were derived for the age and various blood CP parameters. Frequency distribution was calculated for the gender and bone marrow dysplasia.

RESULTS

Age range of the SA patients varied from 14-65 yrs (table-I) and there are more male than (table-II) female. Clinical features included weakness, malaise, easy fatigability, fever, bleeding menifestations, pallor, splenomegaly and syndrome specific features of Wolfram's syndrome

and MELAS (Myoencephalopathy, lactic acidosis and stroke like episodes) in 2 patients. MCV ranged from 66 fl to 94 fl and the dimorphic red cell morphology considered an important feature of the SA was not observed in any patient. Other cytopenias were also noticed particularly thrombocytopenia. Dysplasia was observed in 4 patients including both the congenital SA patients and 2 cases of myelodysplastic syndrome refractory anemia with ring sideroblasts RARS and refractory cytopenias with multilineage dysplasia and ring sideroblasts (RCMD-RS) in large numbers, and in which many levels of metabolic control must coexist. This has resulted in slow pace of research and understanding in the pathophysiology of SA[9]. This study was done to assess the heterogeneity of clinicopathological characteristics of SA. Overall male to female ratio was 1.3:1. All the patients received pyridoxine treatment and there were two males that responded to it indicating the likelihood of underlying X linked ALAS2 gene defect. This finding established the fact that of the remaining eight cases females were actually similarly affected

Table-I: Age and Haematological features of the diagnosed SA patients (n=10).

	Minimum	Maximum	Mean	Std. Deviation
Age	14.00	65.00	28.1000	18.08898
TLC	1.8	11.1	5.330	2.9159
Hb	5.2	10.8	8.050	2.0206
MCV	66	94	76.60	8.682
MCH	21	29	25.20	2.860
Platelets	4	402	149.60	152.002
Valid N (listwise)	1.8	11.1		

Table-II: Gender frequency of the diagnosed SA patients (n=10).

	Frequency	Percentage
Female	4	40.0
Male	6	60.0
Total	10	100.0

Table-III: Frequency of dysplasia in the diagnosed SA patients (n=10).

Dysplasia	Frequency	Percentage
No	6	60.0
Yes	4	40.0
Total	10	100.0

(table-III). One patient was confirmed as having secondary SA due to lead poisoning. The remaining 5 cases were neither tested for molecular lesions nor tested to exclude reversible causes of SA, however as 2 of these patients responded to pyridoxine treatment they may be suffering from the X linked ALAS2 gene defect.

DISCUSSION

Because of its rarity, there have been few clinical and pathological studies focusing on SA. Similarly molecular pathogenetic studies have always been hampered by the fact that the abnormality resides in the dividing and differentiating erythroblast which is difficult to obtain pure and (4:4) as compared to males. One of the affected female patients who was further investigated showed evidence of lead intoxication. Hence the misconception that females are not likely to suffer from SA is wrong and any female in which the anemia fails to respond to standard treatment must be considered for SA investigation. Only the X linked SA is likely to be more common in the males and since X linked ALAS2 gene mutation is the most common cause of inherited SA hence the misconception that SA is a male specific disorder. Theoretically even X linked ALAS2 associated SA may manifest in a female with skewed X chromosome inactivation. The mean age at the

time of diagnosis was 28.1 years and its range varied considerably from 14 to 65 years. All the patients were in a younger age group except for the two MDS associated SA cases who were in older age group. The congenital syndromic SA cases were diagnosed later than they should have been and the main reason for this seems to be lack of familiarity with the disorders by the general practitioners and delay in reaching a tertiary care centre by these patients. Majority of the congenital non syndromic and acquired reversible cases seem to be in the young to middle age as we observed in our patients[10]. The age of onset for the congenital non syndromic SA has been reported to be as early as 01 month to as late as 81 years due to X linked ALAS2 gene defect by Kazumichi Furuyama *et al*[11]. It is important to recognize these entities early in life as specific treatment of these patients may result in avoiding any long-term complications. MDS related SA was observed in the old age. Hence SA is not limited to any age group and the age range for SA generally seems to be from early childhood for the congenital syndromic variants

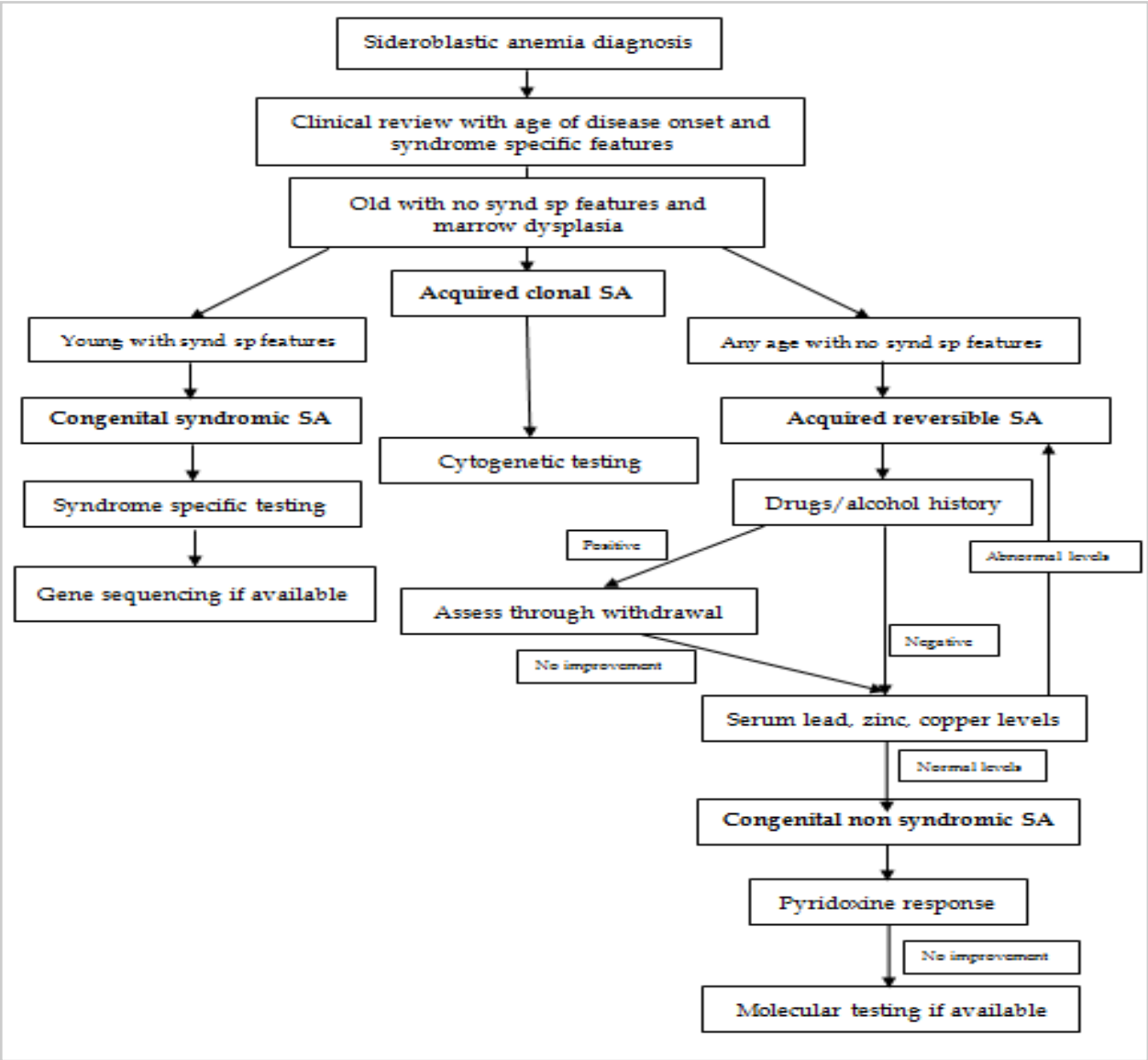

Figure: Flowsheet for the work-up of sideroblastic anemia.

to the old age for the MDS associated cases. All the patients had complaints of weakness, malaise and easy fatigability at the time of presentation. Occasional fever was another very common complaint reported in 6 patients. Petechiae formation, gum bleeding and easy bruising were reported in two of the patients. Among physical findings pallor was common to all the patients and three patients had mild splenomegaly. Although not yet labeled, one of the patients had features of Wolfram's syndrome with hearing disability, early onset diabetes mellitus and decreased vision. No work-up for diabetes insipidus had been done. The patient with MELAS syndrome was bed bound since birth and had physical and mental developmental delay. There was history of myoclonic seizures since 3 years of age. Neurological examination revealed generalized muscle wasting and the limbs were locked in flexed posture. There was global hypo-*tonia with decreased reflexes. Serum lactate level was raised to 32.1 mg/dl. CT scan brain showed cerebral and cerebellar atrophy. Electromyography showed absence of muscle action potentials. Based on the electromyographic evidence of myopathy, history of myoclonic seizures and vomiting, brain atrophy on CT scan, lactic acidosis and sideroblastic anaemia a diagnosis of MELAS was established. He was misdiagnosed as having cerebral palsy effects and was treated for rehabilitation for years before he reached us[12]. The hemoglobin level ranged from 5.2 to 10.8 g/dl. The mean hemoglobin level was 8.05 g/dl which may be an overestimation as three patients had received red cell transfusion prior to these results. Lowest hemoglobin levels were observed in the congenital syndromic SA patients. In different SA types, the anemia is of varying severity and may be associated with the underlying pathophysiology of the disease. Variations in the phenotypic expression even exist within a distinct type of SA[13]. Analysis of full blood counts also revealed that the anemia in SA was associated with other cytopenias particularly thrombocytopenia. Four patients had leucopenia and six had thrombocytopenia in addition to anemia. These included both the congenital syndromic SA patients and one of the MDS patients all three of whom had pancytopenia. The lowest total leukocyte count and platelet count were observed in one of the congenital syndromic SA patient. Only recently the category of RARS-T has been included in the MDS classification where there is JAK2 mutation and this results in thrombocytosis which is in contrast to the cytopenias expected in MDS[14]. Analysis of red cell indices showed that MCV ranged from 66-94 fl. One patient had macrocytosis, three patients had overt hypochromic microcytic indices, five had borderline hypochromia and microcytosis while the remaining one had normocytic normochromic indices. It again goes to show that SA is misconceived to be associated only with hypochromic microcytic indices. The presence of concomitant thalassemia trait was not excluded in the overt hypochromic microcytic cases. The reason why SA is associated with a wide range of MCV just like its association with varied level of haemoglobin may be linked to the underlying heterogeneous pathophysiology. Hence anemic patients without any indices restriction may be investigated to confirm SA. Dimorphic red cell morphology is generally considered to be an important clue to diagnosis of SA. It was not seen in any patient. Once again the explicit association is a misconception as it would only be observed in female patients with X linked disease or RARS. The gold standard for SA anemia is bone marrow demonstration of ring sideroblasts and all our patients had ring sideroblasts in the bone marrow. Except for acquired clonal SA cases i.e. MDS cases where a 'cut off' of 15% was used there is not a clear 'cut off' of ring sideroblasts percentage to give the diagnosis of congenital and acquired reversible SA and frequent observation of ring sideroblasts fulfilling the diagnostic criterion of a ring sideroblast was taken as positive finding for SA diagnosis. As such ring sideroblasts are found exclusively in pathological conditions, and should not be confused with ferritin sideroblasts which are present in normal bone marrow. Both the congenital syndromic SA patients showed

megakaryocytic hypoplasia and dysplasia of the myeloid and erythroid lines indicating a more generalized pathology. Dyserythropoiesis has also been observed in a young patient with hereditary SA by Kyung *et al* from Korea[15]. Congenital dyserythropoietic anemia (CDA) with ring sideroblasts has also been reported by Brien *et al*[16]. Here we propose that CDA be included in the MDS group of diseases as an inherited MDS category with subcategories of CDA and CDA-RS. Dysplasia including dyserythropoiesis was also a feature of the MDS cases however the bone marrow was normocellular to hypercellular in the MDS and all the remaining cases. The importance of early and accurate diagnosis of SA lies in the fact that acquired reversible causes and congenital SA due to ALAS2 mutation are treatable with simple measures while acquired clonal SA requires specialized treatment[17]. Even the congenital syndromic SA patients can avoid unnecessary investigations and frantic roaming from one doctor to the other if their disorder is recognized and the family is offered proper counseling as well as supportive treatment. Increasing knowledge gained from efforts in this area may provide new approaches to treatment. Once SA is identified and categorized, specific treatment can be initiated to address the cause. The importance of precise diagnosis of these disorders using clinical and laboratory evaluation including molecular analysis has been highlighted by Bottomley SS[18]. As SA specific molecular studies are not available in Pakistan it is not possible to precisely identify the congenital and clonal SA disorders however we propose a flow sheet for the diagnostic work up of SA suitable for our country.

CONCLUSION

SA clinicopathological features are variable and this is likely to be a result of its variety of etiologies. It is inappropriate to associate SA with any specific age, gender, MCV range or morphology. Prompt recognition of SA with its accurate categorization and specific treatment can avoid undue suffering of the patients as well as their relatives.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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