The Incidence of Hemoglobin S in Taourga Region, Libya

Mohamed.M.B Marwan [1], Mageda M.Egrouda [2], Nagi A.Jornaz [3], Yosra M.Lamami [4], Housain H. Gadmour [5]

ABSTRACT

This study is to evaluate the incidence of hemoglobin S in Taourga region. Out of 491 blood samples, 62 (12.5%) were abnormal for hemoglobin S [21(4.3%) males and 41(8.4%) females]. Out of 62 samples 57 (12.1%) were heterozygous (HbAS) [18 (4.1%) males and 39 (8.02%) females]. Three samples (1.0%) were homozygous (HbSS) [1(0.2%) male and 2(0.4%) females]. One sample (0.02%) was HbAFS male and one sample (0.02%) was HbAC male. The incidence of hemoglobin S in Taourga region was about 12.5%. Sickle cell disorders are on bases of our results is considered to be a public health problem in Taourga Region, Libya.

INTRODUCTION

Sickle cell disease is one of the commonest genetic disorders worldwide and is the most commonly inherited hematological disease affecting humans (Weatherall and Clegg, 1981) and (Bun and Forget, 1986). The disease is a result from substitution of a single amino acid, valine for glutamic acid in the sixth codon of beta globin gene in chromosome11, causing RBCs undergo sickling and deoxygenated. The susceptibility of red cells to sickle correlates well with the concentration of sickle hemoglobin within the red cells (Koch *et al*., 2000).

The first study of sickle cell anemia in Libya was in Benghazi (East of Libya), and the incidence of HbS was about 3.0% (Jain, 1979). A study of 2095 samples were collected from Wadi Eshati Region, Fezzan showed that 87 samples (4.15%) were found positive for HbS (Marwan, *et al*., 2011).

Sickle cell trait or disease offers a protective effect against malaria in endemic regions, and has led to positive selection for the gene mutation. Its prevalence is 10% to 30% in sub-Saharan Africa, between 25% and 30% of newborns in western Africa are carriers of the sickle cell trait (Haldane, 1948). Prevalence is also high along the southern coast of the Arabian Peninsula, in central and coastal areas of the Indian subcontinent and in Southeast Asia (Howard and Hamilton, 2005).

About 2 million Americans have sickle cell trait and about 1 out of 12 African Americans has sickle cell trait (Hashmi *et al*., 2008). The incidence of sickle cells in Taourga region is 12.5% which is considered to be as high as other studies carried out in other regions in Libya (Marwan, *et al*., 2011).

1. Department of Zoology, Faculty of Science, University of Tripoli, Tripoli, Libya
2. Tamzin hospital, Tamzin, Libya.
3. Department of Anesthesia, Collage of Medical technology, university of Tripoli.
4. Hematologist and Medical Oncologist Tripoli Medical Center, Tripoli, Libya.
5. Cell biology research group, Biotechnology Research Center (BTRC), Twisha, Tripoli, Libya.

MATERIALS AND METHODS

Samples Collection

A total number of 491 blood samples (142 males and 349 females) were collected in a period of four months (July to October) 2009 from Taourga general hospital, Taourga. Five milliliter of venous blood were collected in test tubes contain EDTA as anticoagulant. Samples were labeled and stored at 2-8°C until use not more than two days. Further studies were included Complete Blood Count, Sickling test and Isoelectric Focusing (IEF) electrophoresis.

Complete Blood Count (CBC)

All hematological parameters for each subject were recorded in a strip paper of the Sysmax machine, with time consumed to get a one strip was ranged between one to a half minute

Sickleing test:

One drop of blood sample was taken by a Pasteur pipette, and placed on a glass slide. Two milliliters of sodium metabisalfate was added to the sample and mixed well by a wood rod. The slide was covered by glass cover slip and was pressed will by fingers to get rid of bubbles. After about one hour, the slides were scanned under the light microscope to present either positive or negative result for sickle cell anemia (Al-Nood, *et al*., 2004).

Isoelectric Focusing (IEF) Electrophoresis

Samples were analyzed by isoelectric Focusing (IEF) (Black, 1988) employing gel with a pH range of 6-8 from isolab Inc (Akron, OH., USA)

RESULTS

Sickling test

All samples were run for sickling test to detect HbS. The test was positive in 62 samples and negative in 429 samples, but Sickling test can't distinguish between homozygous and heterozygous of HbS (Figures 1).

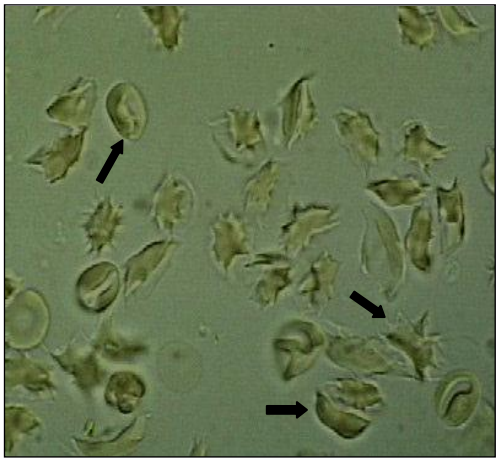

Figure1 Sickling test was done using Sodium metabisulfate (arrows represent the sickled cells).

Molecular Pathology of Sickle Cell disease

Hematological and hemoglobin phenotypes:

Out of the all 491 samples, 62 samples were abnormal for hemoglobin S (12.5%), most of them HbAS (57) and HbSS (3), HbAC (1) and HbAFS (1). Hematological values and hemoglobin composition were given in (Table 1).

Hemoglobin phenotypes:

Out of the 491 participants screened, 62 samples (12.5%) were found positive for HbS. Out of 62 samples, 57 samples (12.00%) were heterozygous (HbAS), one sample HbAC (0.20%), one sample HbAFS (0.20%), and three samples (1.00%) were homozygous for HbS (Hb SS), (Table 2).

Tables 1 Hematological data of sickle cell patients and hemoglobin type.

Case	Gender-(year)	RBCx 10^{12}/L	Hb g/dl	MCV fL	MCH pg	Sickling test	Hb Type
*LA 05	F-21	3.21	12.6	97.1	31.1	Positive	Hb AS
LA 09	F-22	4.12	11.9	84.8	29.3	Positive	Hb AS
LA 15	M-29	4.67	14.2	86.7	31.4	Positive	Hb AS
LA 16	F-34	5.36	12.8	68.4	22.9	Positive	Hb AS
LA 20	F-30	4.67	11.7	72.1	19.8	Positive	Hb AS
LA 22	F-25	4.23	8.2	64.2	18.9	Positive	Hb AS
LA 24	F-27	6.08	11.5	93.9	27.7	Positive	Hb AS
LA 34	F-65	3.56	12.6	97.2	30.4	Positive	Hb AS
LA 57	F-60	4.72	12.3	83.9	29.8	Positive	Hb AS
LA 63	F-48	4.83	14.1	87.4	30.2	Positive	Hb AS
LA 64	M-50	3.24	12.8	90.3	33.4	Positive	Hb AS
LA 71	F-33	5.32	11.2	88.5	29.7	Positive	Hb AS
LA 74	F-27	6.21	11.3	67.9	19.5	Positive	Hb AS
LA 92	M-40	5.83	12.1	92.4	30.8	Positive	Hb AS
LA 93	F-25	3.43	12.4	68.7	21.4	Positive	Hb AS
LA 94	M-29	3.75	9.4	75.2	25.1	Positive	Hb SS
LA 95	F-18	2.56	10.8	68.9	28.3	Positive	Hb SS
LA 103	F-46	2.97	11.5	73.5	19.9	Positive	Hb AS
LA 104	F-75	5.10	10.9	67.6	17.8	Positive	Hb AS
LA 117	M-70	6.41	12.3	74.6	30	Positive	Hb AS
LA 122	M-61	5.23	13.4	81.3	31.2	Positive	Hb AS
LA 124	F-25	3.47	11.7	90.2	33.1	Positive	Hb AS
LA 130	M-20	4.28	14.6	88.4	31.43	Positive	Hb AS
LA 136	M-64	3.32	11.9	78.6	38.5	Positive	Hb AS
LA 140	M-11	3.56	12.3	80.1	29.7	Positive	Hb AS

LA 143	M-17	2.49	7.5	67.4	21.3	Positive	Hb AS
LA 147	F-39	5.31	13.8	90.3	30.1	Positive	Hb AS
LA150	F-30	3.23	9.2	69.1	23.4	Positive	Hb AS
LA198	F-28	4.82	12.2	80.4	29.6	Positive	Hb AS
LA 202	M-7	2.51	10.2	78.4	23.5	Positive	Hb AC
LA 212	F-47	4.14	10.8	90.2	30.3	Positive	Hb AS
LA 214	F-45	3.34	9.9	81.8	29.4	Positive	Hb AS
LA 220	F-32	5.27	12.5	65.7	20.3	Positive	Hb AS
LA 222	M-2	2.45	8.8	91.1	30.3	Positive	Hb AFS

*LA= Libyan Adult

Table 2 The incidence of hemoglobin variants.

Hemoglobin type	**number**	**Percentage (%)**
HbAS	57	12.00
HbSS	3	1.00
HbAFS	1	0.20
HbAC	1	0.20
Total	62	12.5

Out of the 62 samples that showed positive for HbS, 21 samples males (4.30%) were positive for Hb variants (HbAS, HbSS, HbAFS, HbAC). Out of these, 18 samples (4.10%) were heterozygous (HbAS), one sample (0.20%) HbAC, one sample (0.20%) HbAFS and three sample (1.0%) HbSS (Table 2). Forty one females (8.40%) were found positive for (HbAS, HbSS). Out of which 39 samples (8.02%), were found heterozygous (HbAS), and 2 samples (0.40 %) were homozygous (HbSS) (Table 3).

Table 3 The incidence of hemoglobin variants among males & females of the total samples.

Sex	**HbAS**		**HbSS**		**HbAFS**		**HbAC**		**Total**	
	N	%	N	%	N	%	N	%	N	%
Males (N =142)	18	4.10	1	0.20	1	0.20	1	0.20	21	4.30
Females (N=34	39	8.02	2	0.40	0	0%	0	0	41	8.40
Total (N=491)	57	12.00	3	1.00	1	0.20	1	0.20	62	12.70

Isoelectric focusing (IEF) electrophoresis:

Comparing the two methods that were run in this study (the sickling test and the IEF test) to detect HbS. It was found that the sickling test gave the same results as compared to IEF test. The sickling tests can't distinguish between

homozygous and heterozygous of HbS as the IEF test. IEF test was considered to be more accurate for the detection of HbAS, HbS, HbC, HbA, HbF and HbA2 which were precipitated on the gel as clear bands (figure 3&4).

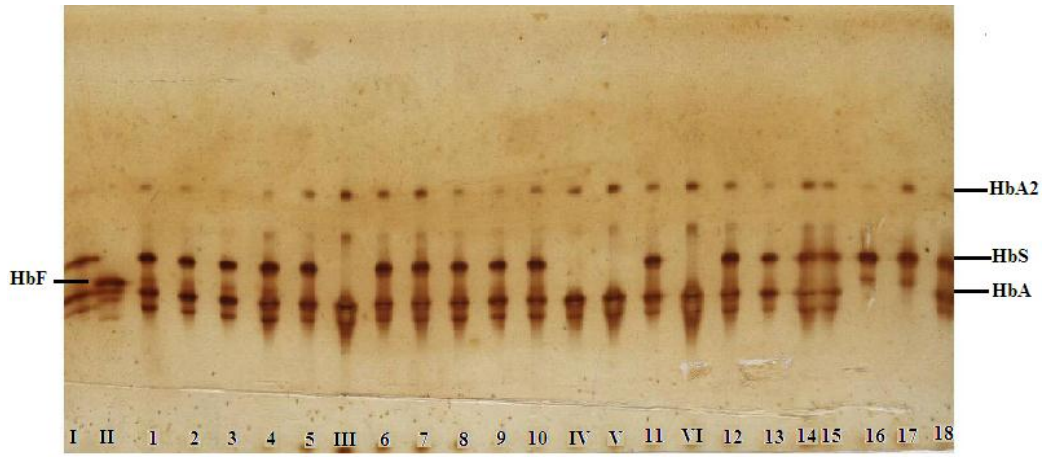

Figure 3: IEF gel (Sample No. :I HbAS control. Sample No. :II HbF control. Sample No: III, IV,V, VI HbA control). (Sample No: 1 to 15 and 18 positive HbAS, While sample No: 16 and 17 HbSS).

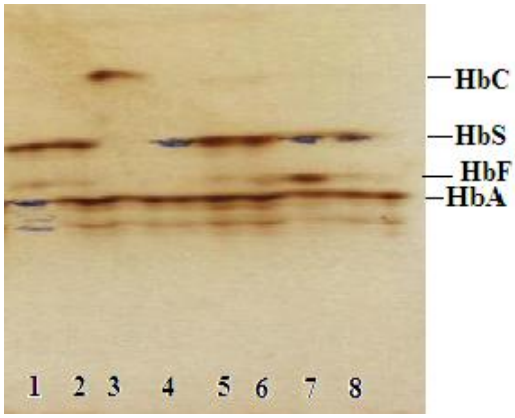

Figure 4: IEF gel (Samples No: 1, 2, 4, 5 6 and 8 HbAS, sample No: 3 HbC. Sample No: 7 HbAFS

Discussion

This study was carried out from July-October 2009 on 491 blood samples from the Taourga region. The racial origin of this assessed population was mostly Arab, with a mixture of African Arab, Berber minority and sub-Saharan African. Several abnormal hemoglobin genotypes were identified during this study; these were HbAS, HbSS and Hb AC. The overall gene incidence of the hemoglobinopathies in the region of Taourga was 12.5%.

The people of Taourga were restricted to intermarriage between themselves. HbS gene frequency was very high in the Taourga region (12.5%). This could be explained by the fact that the Taourga population consists of a mixture of Arab, Africans, Berbers and sub-Sahran Africans. The incidence of HbS was shown to be high in the Taourga Region. The incidence of sickle cell anemia in different parts of Libya by other studies was found to be in Benghazi was 4.2% (Jain, 1979), and 4.2% (Marwan, 1998).

On the other hand, most of the HbS positive samples were heterozygous (12.5 %), whereas, the prevalence of HbSS was 1.0%, and this is confirmed by many other similar studies and this percentage

is also high when compared to other studies in different parts of Libya which demonstrate that the HbAS was 1.6%, and 0% for HbSS (Marwan, 1998).

The incidence of HbAS is also higher when compared to other studies in Wadi Elshati region. The 2095 samples screened, 87 samples (4.15%) were found positive for HbS. On the other hand, 83 samples (3.96%) were found heterozygous for HbS (HbAS), and 4 samples (0.19%) were homozygous for HbS (HbSS) (Marwan, *et, al*., 2011).

The incidence of HbAS is also higher when compared to other studies in Yemen. The 1700 were screened, among them 37 samples (2.2%) were Heterozygotes (Al-Nood, *et al.*, 2004).

The incidence of HbAS was lesser than when compared to other studies in Nigeria. The 3600 samples were collected at Kano general hospital in Nigeria, 1297 samples (36.0%) were Heterozygotes for HbAS, and 1589 samples (44.1 %) were homozygous for HbS (HbSS) (Weatherall and Clegg, 1981). Results from a study in Uganda showed that 46% of the population may have sickle cell trait Hb AS, and 2% have Homozygotes HbSS (Nelson, *et al*., 2003).

According to the tests carried out in this study, It has been found that sickle test was compatible with Isoelectric focusing tests in the detection of HbS. This test may be used as primary screening tests, on account of low cost, but it can't differentiate between HbAS and HbSS in contrast with IEF test. On the other hand, sickle test is not sensitive for the detection of HbAC (Deshmukh *et al*., 2006).

Conclusion

The incidence of sickle cells in Taourga region (12.5%) which is almost higher to those of other studies carried out in other regions in Libya. Sickle cell disorders are on bases of our results considered a public health problem in Taourga Region. A systematic neonatal screening programme for this disorders and all other abnormal hemoglobin seems reasonable. Adapted management of hereditary sickle cell should be available as early identification, control and management of sickle cell disorders is necessary to prevent childhood deaths.

REFERENCES

1. Al-Nood H, Al-Ismail S, King L, and May A; Prevalence of the Sickle Cell Gene in Yemen: A Pilot Study*. Hemo. J*. **28(4)**: 305–315, 2004.
2. Black, J. An isoelectric Focusing method to Detect hemoglobin variants in newborn blood samples the β-thalassemia. Hemoglobin, 12: 681-690, 1988
3. Bun, H.F and Forget, B.G.: Hemoglobin: Molecular, Genetic and clinical Aspects, W.B Saunders, Philadelphia, PA, 1986.
4. Deshmukh P., Garg B S., Garg N., Prajapati N C., and Bharambe M S: Prevalence of Sickle Cell Disorder in Rural Wardha. *Ind J of Comu Med*. **31**: 26–27, 2006.
5. Haldane, J.B.C.: The rate of mutation of human genes. Proc. Eighth Int. Congr. Genet, pp267-273(Hereditas Supl.35), 1948.
6. Hashmi K.N, Moiz B, Nusrat M, and Hashmi M.N; Chromatographic analysis of HbS for the diagnosis of

various sickle cell disease in Pakistan. *An of Hemato.***10**:1432-1439, 2008.

7. Howard M.R, and Hamilton P.J; Sickle cell syndrome. Hematology an illustrated color text. E Lsevier Churchill Livingstone. 2nd edi. 34–35, 2005.

8. Jain R C: Hemoglobinopaties in Libya. *J Trop Med Hyg*. **82**: 128-132, 1979.

9. Koch A, Yang Q, and Olney R S; Sickle Hemoglobin Allele and sickle cell diseases. *A. J. Of Epid.* **151(9)**: 839-845, 2000.

10. Marwan M.B; Molecular Epidemiology of hemoglobin in the population of Libya and the molecular biology of normal and abnormal globin gene expression. Thesis submitted to Degree of Doctor Philosophy. University of Malta 1998.

11. Marwan, M. M., Abdulsalam A. J.,and Abubaker M.M. Prevalence of hemoglobin S in Wadi Eshati Region. Journal of Sabha University (Pure and Applied Science). **10(2)**: 33-42, 2011.

12. Nelson, JB, DPM Brandjes, *et al*. Sickle cell disease; a general overview. *The Netherlands Journal of Medicine*. **62**: 364-371, 2003.

13. Weatherall.D.J and Clegg, J.B.: The thalassaemia syndromes, 3rd edition. Blackwell Scientific Publicatoins, Oxford, 1981.