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Clinical and Laboratory Features of Sickle Cell Disease S/D Punjab: Impact of HbF and Hydroxyurea

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Abstract. ***Background*: Sickle cell disease (SCD) is a major public health issue worldwide with high morbidity and mortality. SCD SD Punjab is the third most common genotype of SCD in Oman and is associated with several serious complications. The aim of the study is to establish the clinical and laboratory features of SCD patients with SD double heterozygotes and study the impact of haemoglobin F, hydroxyurea, and other modulators on the disease severity.**
***Methods*: We analysed the electronic medical records of 52 consecutive SCD patients who were diagnosed as double heterozygote SD Punjab between 2006 and 2022. The study was approved by the local medical research and ethics committee. The data captured included SCD-related complications and current clinical and laboratory indices. Data from other studies on other SCD genotypes were used as historical controls.**
***Results*: 52 patients (31 males, 21 females) who formed this cohort had a median age of 32 years with an interquartile range (IQR) of 21-39.8 years. 37(71.2%) had <3 VOC per year, whereas 15 (28.8%) patients had ≥3 vasooclusive (VOC) episodes per year. SCD-related complications included Acute Chest Syndrome (ACS) (48%), Gall stones (26.9%), Avascular necrosis (AVN) (28.8%), Stroke (13.5%) and splenic sequestration (7.7%), whereas 5 (9.6%) patients of this cohort died. Surgical and Autosplenectomy were seen in 18 (34.6%). These findings were similar to other SCD genotypes in this community.19 (57.6%) were taking Hydroxyurea (HU) amongst the 33 patients who were prescribed HU. Haematological parameters showed a median (IQR) Hb (g/dl), MCV (fl), Retic count (%), WBC count($X10^9$/L) and Platelet count($X10^9$/L) of 9.7 (8.5-11.3), 74.9 (68.4-79.8), 4 (3.2-5.7), 9.9 (8.1-12.6) and 309 (239-428) respectively. The haemoglobin electrophoresis showed an elevated HbF, whereas serum bilirubin and LDH were elevated amongst the biochemical parameters. The use of hydroxyurea showed no impact on VOC, ACS, AVN, Stroke or mortality.**
***Conclusion*: SD Punjab is the third most common SCD genotype in Oman and was associated with recurrent VOC, ACS, AVN, and gall stones comparable to other SCD genotypes. Patients with > 3 VOC/year had significantly increased incidence of Stroke, AVN, and gallstones. However, HU was not associated with improved prognosis and better survival in this cohort of patients.**

Keywords: Sickle Cell Disease; Haemoglobin SD Punjab; Vaso-occlusive crises; Acute chest syndrome; Avascular Necrosis; HbF; Hydroxyurea.

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Introduction. Sickle cell Disease (SCD) is an inherited, autosomal recessive, monogenic, multi-systemic blood disorder with the highest prevalence in sub-Saharan Africa and the Middle East.[1-2] It is characterized by a single amino acid mutation, where glutamic acid is replaced by valine in position 6 of the beta chain of haemoglobin molecule.[3,4] The hemoglobin-S polymerizes in the post-capillary venules, causing the red blood cells to assume a distorted, sickle-like shape, first described by James Herrick in 1910.[5] Haemoglobin D Punjab, also known as Hb-D Los Angeles, is formed by a missense mutation in the beta globin gene, resulting in a substitution of the amino acid (Glutamate) into (Glutamine) at the 121st position of beta chain[6,7] and is the third commonest variants after HbS/S and Hb S/β in Oman.[2]

The clinical course of S/D Punjab heterozygotes is characterized by recurrent vaso-occlusive crises (VOC), chronic hemolytic anaemia with gall stones, acute chest syndrome (ACS), avascular necrosis (AVN) of the hips similar to homozygous Hb SS.[8-10] Hemoglobin-SD disease is associated with mild to moderate splenomegaly. Although the level of HbF and the use of hydroxyurea (Hb F inducer) does influence the severity of SCD, there are contradicting reports of the impact of HbF on disease severity and symptoms of SD-Punjab patients.[9,11]

Since the literature on Hemoglobin SD Punjab is limited, this is the first comprehensive single-centre study to report the clinical presentations, complications, and outcomes of SCD—Hb SD Punjab in Oman and understand the impact of alpha thalassemia, haemoglobin F, and hydroxyurea on the disease severity in these SCD patients.

Materials and Methods. In this retrospective study, 52 consecutive SCD patients who were diagnosed as Hemoglobin S/D Punjab Heterozygotes between 2006 and 2022 at the Sultan Qaboos University Hospital in Oman were enrolled, following written approval from the local medical research and ethics committee (MREC # 2551). The data was obtained from the electronic patient record system and included demographic features SCD related manifestations such as the frequency of painful vasooclusive crises, ACS, splenic sequestration, AVN, priapism, gallstones and Stroke. We also analyzed the haematological, biochemical and radiological parameters, as well as the use of hydroxyurea therapy. The laboratory results obtained included complete blood counts, haemoglobin electrophoresis data, and alpha thalassemia status (when available). Biochemical parameters included renal and liver function tests along with serum LDH levels. We utilized data from historical controls of our previous studies relating to other genotypes of SCD in our population.

Statistical Analysis. The statistical package for social science (IBM SPSS, USA ver.23, Armonk, NY) was used to analyze the collected data. Normally distributed data was characterized as mean with standard deviation, whereas data that was not normally distributed was characterized as median with interquartile range (IQR) for continuous variables and percentage and frequency for categorical variables. Clinical and laboratory parameters were compared using the means for continuous measures and tested for association using the Student t-test, Fisher's exact test and chi-square test. A P value of <0.05 was considered to be statistically significant.

Results. Amongst the 52 SCD SD Punjab double heterozygotes, 31 (59.6%) were males with a median age (IQR) of 33 (21-41) years and a range between 14 to 46 years (**Table 1**). 31% of this cohort were below 25 years old, whereas the age of majority (69%) ranged between 25 to 46 years. There was no gender preponderance for the SCD manifestations like VOC events, ACS, splenic sequestration or Stroke and mortality in this cohort, but splenic atrophy/splenectomy was more common in females (p=0.007, Fisher's exact test).

Table 2 shows no significant differences when a historical cohort of other SCD genotype patients that are followed at our centre were compared with our current cohort of SD SCD patients. These are not head-to-head comparisons but historical data of other SCD genotype patients from previous publications.[12]

Recurrent VOC events were recorded in all the patients, with 71.2% showing less than 3 episodes per year and 28.8% showing 3 or more episodes per year. Many of these patients had a number of acute sickle cell-related complications, including ACS, Stroke, and splenic sequestration in 48%, 13.5% and 7.7%, respectively. Stroke was more common in the patients with VOC episodes >3/year (p=0.007, Chi-square test). On the contrary, ACS was more frequent in patients with VOC episodes <3/year, but this association was not statistically significant (p=0.02, Chi-square test) (**Table 3a**).

Further, chronic complications such as AVN and gallstones were seen in 28.8% and 26.9%, respectively. AVN and Gall stones were more prevalent in patients with VOC episodes > 3/year, and this association was statistically significant (p=0.01 and p=0.006, respectively, Chi-square test). Most patients had normal spleen (57.7%). However, absent spleen was confirmed in 34.6% (Auto or surgical splenectomy), whereas 7.7%

Table 1. Demographic, clinical and laboratory parameters in SCD SD Punjab cohort (n=52).

Parameters	Results	Cases
Age in years, Median (IQR)[Range]		32(21-39.8) [14-46]
Gender, Total cases, n, M: F		52(31:21)
Males, age in years, Median (IQR)		33(21-41)
Females, age in years, Median (IQR)		31(20.5-38)
Blood Group (n=42)		
	A POS, n (%)	21(40.4)
	B POS, n (%)	10(19.2)
	Other groups, n (%)	11(21.2)
Past medical history		
VOC	VOC 1-2/year, n (%)	37(71.2)
	VOC ≥3/year, n (%)	15(28.8)
ACS, n (%)	ACS, n (%)	25(48)
Stroke, n (%)	Stroke, n (%)	7(13.5)
Splenic sequestration, n (%)	Splenic sequestration, n (%)	4(7.7)
Priapism, n (%)	Priapism, n (%)	0(0)
Gallstones, n (%)	Gallstones, n (%)	14(26.9)
AVN	No AVN, n (%)	37(71.2)
	AVN, n (%)	15(28.8)
	Unilateral AVN, n (%)	3(5.7)
	Bilateral AVN, n (%)	8(15.4)
	AVN Hips+ Shoulders, n (%)	3(5.7)
Hydroxyurea,	Hydroxyurea, n (%), [31 patients prescribed HU]	19(57.6)
Spleen Status	Spleen Status-Normal, n (%)	30(57.7)
	Splenomegaly, n (%)	4(7.7)
	Splenectomy- Auto & Surgical, n (%)	18(34.4)
Deaths, n (%)	Deaths, n (%)	5(9.6)
Laboratory Parameters, Median (IQR)		
Red cell parameters	Haemoglobin, g/L	9.7(8.5-11.3)
	MCV, fL	74.9(68.4-79.8)
	MCH, pq	25.6(23.3-27.4)
	Retic Count, %	4(3.1-5.7)
WBC	WBC counts basal,10^9/L	9.9(8.1-12.6)
Platelet counts	Platelet counts basal, X10^9/L	309(239-428)
HPLC results	HbF, % (IQR), [Range]	8(3.4-15.5),[0.5-38.1]
	HbD, % (IQR),[Range]	45(43.1-46.5),[36.3-51]
	HbS, % (IQR),[Range]	38.6(33.4-43.3), [20.4-49.5]
Biochemical results	LDH, IU	475(373-687)
	AST, u/L	37(27-51)
	ALT, u/L	20(13-27)
	ALP, u/L	103(71-180)
	Serum Bilirubin, mg/dl	26(21-48)
	Serum Creatinine, %	49(40-60)

Key: IQR- Interquartile Range; VOC-Vasoocclusive crisis; ACS-Acute Chest Syndrome; AVN-Avascular Necrosis; HPLC -high performance liquid chromatography, LDH-Lactic Dehydrogenase; AST- aspartate transaminase; ALT- alanine transaminase; ALP- alkaline phosphatase.

had splenomegaly. Further, there was a significant association of splenic sequestration with splenectomy (p=0.002, Fisher's Exact test). Lastly, although 59.6% were males, no case of priapism was reported in this cohort. The most common blood group was A+ (40.4%), followed by B+ (19.2%), O+ (13.4%), O- (5.7%), and AB+ (1.9%).

Amongst the haematology parameters, the median (IQR) Hb (g/dl), MCV (fl), Retic count (%), WBC count (X10^9/L) and Platelet count (X10^9/L) were 9.7(8.5-11.3), 74.9 (68.4-79.8), 4 (3.1-5.7), 9.9 (8.1-12.6) and 309 (239-428) respectively. However, leukocytosis and thrombocytosis were present in 32% and 26% of this SD-Punjab cohort, respectively, with thrombocytopenia present in 10% of the group.

Haemoglobin variant analysis revealed that haemoglobin S and haemoglobin D were the two prominent variants in these Hb SD Punjab patients. Further, it was observed that as the HbF fraction increased, the level of HbS decreased in these patients. Additionally, data on the ^{BS} genotype, Alpha genotype, and Sickle Haplotype details were available, albeit in a small proportion of patients in this cohort only.

The biochemical analysis of liver function tests, creatinine and LDH were found to correlate with disease activity, with the majority showing normal liver functions but raised LDH and serum bilirubin levels and low serum creatinine levels. The interrelationship

Table 2. Comparison between SCD SD Punjab and SCD HbSS (historical controls-ref[$]).

Parameters	Cases (52)	Controls (67)	P value
Median age in years	32	31	0.36[#]
Median age in years, males	32	27	0.18[#]
Median age in years, females	31	31	0.41[#]
Past History			
VOC 1-2/year	37 (71)	48	1.0[#]
ACS, n (%)	25(48)	39(58.2)	0.54[#]
Gall stones	14(26.9)	15(22.4)	0.65[#]
AVN	15(28.8)	9(13.4)	0.09[#]
Asplenia	18(34.4)	28(41.8)	0.59[#]
Laboratory parameters			
Median Hemoglobin g/dl	9.7	9.2	NS[@]
Reticulocytes	4	6.3	NS[@]
Media WBC count, basal	9.9	11.3	NS[@]
Platelets counts basal	309	333	NS[@]
Median Hb F, %	8	8.5	NS[@]
Median LDH, IU	475	415	NS[@]
Median AST, uL	37	38	NS[@]
Median ALT, uL	20	30	NS[@]
Median S bilirubin, mg/dl	26	35	NS[@]

Key: [#]Chi Square test; NS[@] Students T-test- p-value >0.05.
Ref[$] - Int J Infect Dis. 2021 Mar 16; 106 :128-133.

Table 3a. Interrelationship between Clinical Parameters and VOC episodes (n=52).

	VOC 1-2/ Year (n=37) (%)	VOC >3/ Year (n=15) (%)	P value
Stroke(n=7)	2(28.6)	5(71.4)	**0.007[#]**
ACS(n=25)	14(56)	11(44)	**0.02[#]**
Splenic Sequestration(n=4)	2(50)	2(50)	**0.33[#]**
Gall Stones(n=14)	6(42.9)	8(57.1)	**0.006[#]**
AVN (n=15)	7(46.6)	8(53.4)	**0.01[#]**
Alpha Thal (n=8)	1(12.7)	7(87.5)	**<0.001[#]**
Median Hb ±SD g/dl	9.7 ± 1.7	10.2 ± 1.2	**0.39[##]**
Mean±SD MCV, fl	73.4±7.1	80.5±8.4	**0.05[##]**
Mean±SD HbF, %	10.3±9.1	10.5±7.5	**0.87[##]**
WBC x 10^9/l ± SD	9.7 ± 3	11.4 ± 3.3	**0.10[##]**
Bilirubin mg/dl, ± SD	39.6 ± 5.6	29.8 ± 6.7	**0.20[##]**
LDH IU, ± SD	576 ± 186	505 ± 180	**0.40[##]**

Key: [#] Chi Square Test; [##]Students T test

severity of vasoocclusive crisis and outcome parameters is reported in **Table 2a**, which shows comparable levels of Hb and also HbF, as well as WBC, between those with mild disease vs. moderate to severe. Similarly, there were statistically significant differences in patients with Stroke, gall stones, presence of alpha thalassaemia and AVN (Chi-Square test). The study did not find any statistically significant association between HU therapy and VOC episodes, ACS, Splenic sequestration, AVN, Gall stone, Stroke or Mortality (**Table 3b**). However, there was a statistically significant difference in patients who took HU with a higher mean MCV and MCH values (Students t-test).

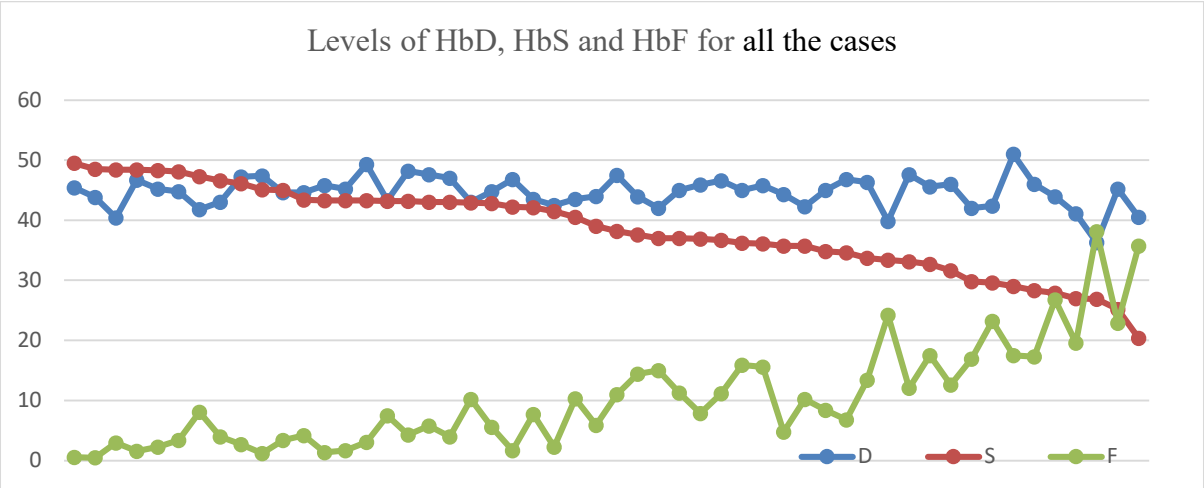
Discussion. Compound heterozygosity of HbS and other haemoglobin variants like HbC, HbE, and HbD gives a great clinical and haematological heterogeneity within SCD[4]. Although HbS/D Punjab has a multicentric origin, it is highly prevalent in the Punjab region of India and Pakistan.[8] Hemoglobin-D does not cause sickling but

Table 3b. Correlation of Clinical Parameters with Hydroxyurea Therapy (n=33).

		Hydroxyurea therapy (n=19)	Without hydroxyurea (n=14)	P value
VOC	**1-2/Year**	11 (57.9%)	9 (64.3%)	**P = 0.60**
	≥3/Year	8 (42.1%)	5(35.7%)	**P = 0.70**
Stroke		4(21%)	3 (21.4%)	**P = 0.97**
ACS		10 (52.6%)	9 (64.3%)	**P = 0.50**
AVN		10 (52.6%)	3 (21.4%)	**P = 0.06#**
Alpha Thal		5(26.3)	1(7.14)	**P = 0.15**
Hb g/dl, ± SD		10.2 ± 1.2	10.1 ± 1.6	**P = 0.90**
Mean±SD HbF %		10.5±7.2	9.2±7.1	**P = 0.45**
Mean±SD HbS %		37.9±6.9	39.5±7.5	**P = 0.25**
Mean±SD HbD %		43.6±4.1	44.9±1.6	**P = 0.35**
Mean±SD MCV, fl		77.9±7.4	70.2±8	**P= 0.01##**
Mean±SD MCH, pg		26.6±2.6	24.4±2.8	**P= 0.03##**
Mean±SD AST, u/L		31.7±12.3	45.1±18.5	**P= 0.02##**
Bilirubin mg/dl ± SD		29.8 ± 18.6	41.5 ± 15.7	**P=0.10##**
LDH IU ± SD		505 ± 186	513 ± 228	**P=0.80##**

Key: # Chi Square Test; ##Students T test.

Figure 1. Median Hemoglobin S, HbD and HbF levels in the current 52 SD Punjab Study Cohort.



has an electrophoretic mobility indistinguishable from that of hemoglobin-S.[13] Although Hb D trait and Homozygous D are relatively asymptomatic, with no underlying haemolytic anaemia, in compound heterozygous Hb SD Punjab disease, Hb D Punjab interacts with Hb S to enhance polymerization and sickling process and produces moderately severe sickle cell disease phenotype and hemolysis.[10] SCD is a heterogeneous disease with variable clinical and laboratory expression due to multiple genetic and environmental factors.[14] Adekile A. et al. in 2010 reported that Hb-SD disease in patients from Kuwait was associated with mild to moderate splenomegaly, recurrent hemolytic anaemia, acute chest syndrome, and also AVN of the hips.[9] Further, Al-Barazanchi et al., in their report on 42 patients from Iraq, observed that recurrent vaso-occlusive events were similar to sickle cell anaemia, and the clinical course was indistinguishable from homozygous Hb SS.[10] In keeping with the above literature, we evaluated 52 patients with SD, finding that recurrent VOC events were seen in all these patients, with moderate to severe VOC episodes (>3/year) that were seen in about a third of this cohort (28.8%). Among the group with severe recurrent VOC (>3 /ear), we found them more likely to have Stroke, Gall stones and AVN, although the level of Hb F was similar in both groups (**Table 3a**). Also, our cohort showed significant other complications, including ACS in 48%, Stroke in 13.5% and splenic sequestration in 7.7%. Among the chronic complications of SD, AVN and gallstones were present in 28.8% and 26.9%, respectively. These findings were similar to those of other SCD genotypes within our community, as indicated in **Table 2**. The impact of HbF is not clear and

raises the question about the lack of protective effect in this group of patients, a point that was also raised previously.[9] Similarly, among the AVN group, hip joints were the most affected, but shoulders were also involved. Also, these patients have lost their spleen (surgical and autosplenectomy) in about 35% of patients, which is similar to reports in other SCD genotypes from our region.[15]

The laboratory data showed that the Hb Level, WBC, and platelets were similar to what is expected in patients with SS and Sβ^o with normal renal and hepatic functions. Also, patients showed a relatively high HbF generally, with and without hydroxyurea, and a decline in Hb S level, with rising HbF, with the Hb D level remaining constant.

Further, the laboratory findings reveal a basal Hb for this cohort of 9.7 g/dl, which is generally similar to what is expected for other SCD groups. Values for WBC and platelets are also as expected. However, basal HbF is relatively high at 8%, and it was as high as 38% in some cases. Amongst the 33 patients on HU therapy, as expected, patients had a higher MCV & MCH (statistically significant), with higher Hb F; however, it did not have a significant impact on the frequency of VOC, Stroke or ACS. Although there is a trend towards the development of AVN, it did not reach statistical significance and justify the earlier comment about the protective role of hydroxyurea and HbF in SD-related complications (**Table 3b**). Interestingly, among the 19 patients on HU, 4 patients (21%) had Stroke. Further, although 57.6% were taking HU, ACS was seen in a proportionately higher number of patients (75%) than those who did not take HU (53%). However, this difference also was not statistically significant. Furthermore, the subset of patients with increased severity in the HU group, represented by >3 VOCs/year, had a significantly higher MCV and MCH, indicative not only of an effect of HU but also of a probable role of the high incidence of alpha thalassaemia, that has been reported in the Omani population and is known to ameliorate the severity of VOC's.[16,17]

In contrast to the HbSS patients who with a high HbF had a milder clinical severity Haemoglobin SD compound heterozygotes from Kuwait with a higher HbF demonstrated a severe clinical course.[9] This reflected the high prevalence of Arab-Indian haplotype in the Kuwaiti SCD population.[9] Further, it also appeared that the interaction of HbD and HbF was unable to prevent or inhibit the polymerization of HbS in spite of the high HbF levels, nor did it ameliorate the clinical course of SCD in this population. In our cohort, although the IQR of HbF ranged from 3.4-15.5% with a median of 8%, the actual range of the literature cases was from 0.5% to 38.1%. This also reflects the mixed SCD haplotypes of Benin, Bantu, and Arab-Indian reported in our SCD population.[18] In the small subset of these patients where data of the sickle haplotypes were available, the mean HbF (SD) in the Bantu haplotype was 1.8±1.1, with only one-third of patients on HU, while, in the Arab Indian haplotype, the mean (SD) was 13.4±5.7 with two-thirds of the patients taking HU, whereas, in the Benin haplotype, the mean HbF (SD) was 13.5±3.3 with all the subgroup patients taking HU. Thus, since the number of subjects was small, any further statistical analysis /conclusions were not possible. Nevertheless, this study did not find any significant association between HU therapy and VOC episodes, ACS, Splenic sequestration, AVN, Gall stone, Stroke or mortality.

Interestingly, we found that the most common blood group in this cohort was A+(40.4%), whereas the most recent study from the country revealed that A+ was only seen in 17.4%, while the most common blood group reported from Oman was O+ (44.9%) followed by B+ (20.2%), A+ (17.4%), AB+ (6.8%), B- (2.7%), O- (7.4%), and A- (0.6%).[19] It is difficult to explain this observation, and a larger sample size is needed to make comparative observations. Nevertheless, a founder effect may explain this observation.

Conclusions. Although this is a retrospective study of the SCD-SD Punjab genotype, it is one of the largest single-centre studies. It demonstrated that SD disease is associated with significant morbidity and mortality, similar to other SCD genotypes. It also demonstrated that although Hb F is high, and with the use of HU, its impact on disease morbidity and mortality needs to be clarified, and longer follow-up on a larger sample of these patients is needed. Although this study is the largest single-centre study of the SCD SD genotype, however, it is a retrospective study, and the sample size is relatively small. Thus, more collaborative prospective work is needed to define the precise clinical and laboratory features of this specific SCD genotype.

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Author Contributions. All authors have made substantial contributions, have seen, and approved the final version of manuscript. SAK and AVP were fully involved in the conception and design of the study, recruitment and care of patients, acquisition of data, analysis and interpretation of data and was instrumental in the drafting the article and critical appraisal before submission.

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