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Original Research Article

Prophylaxis oral aspartame improves anaemia and decreases frequency and severity of vaso-occlusive painful crisis among sickle cell disease patients and noninferior to hydroxyurea

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ABSTRACT

Background: Sickle cell disease (HbSS) causes much of the morbidity and mortality due to its chronic anaemia and vaso-occlusive painful crisis. Hydroxyurea is now using to treat HbSS. Aspartame appears to have antisickling properties. The objective of this study was to asses the effects of prophylaxis oral Aspartame among HbSS patients on anaemia and vaso-occlusive painful crisis.
Methods: 208 cases of HbSS patients with history of vaso-occlusive crisis (VOC) and/or anaemia were selected for the study and divided into study group (n = 104) and control group (n = 104). 31 and 38 patients were on Hydroxyurea in study group and control group respectively. Baseline hemoglobin was estimated in all cases and oral Aspartame was given in a dose of 4 mg/kg/day in three divided doses. Monthly hemoglobin levels were measured in all cases for six months. Data were collected and compared with control data by using SPSS software version 25.
Results: Mean baseline hemoglobin was 6.99 gm% and increased to 8.43 gm% (P <0.05) after receiving Aspartame prophylaxis. Aspartame add on to Hydroxyurea also had increased in haemoglobin levels. Incidence of VOC was 7.26% in control group. In Hydroxyurea group 3.58% had VOC. In study group 1.2% and in add on to Hydroxyurea group had 1.125 of milder degree of VOC (P <0.05).
Conclusions: Aspartame appears to have antisickling properties and improves anaemia and decreases frequency and severity of VOC among HbSS patients and is non-inferior to Hydroxyurea.

Keywords: Anaemia, Aspartame, Prophylaxis, Sickle cell disease

INTRODUCTION

Sickle cell hemoglobinopathy is most commonly encountered in the western part of Odisha, (India) causing much of the morbidity and mortality due to its vaso-occlusive painful crisis and protean clinical manifestation of anemia.[1,2] Sickle cell haemoglobin result from a point mutation of substitution of valine for glutamic acid in the 6th position of β-chain of haemoglobin called Sickle haemoglobin (HbS). This leads to increase polymerization of HbS on deoxygenation. Its homozygous state is called sickle cell disease (HbSS). Sickle cell vaso-occlusive crisis and chronic anaemia is more common in HbSS patients.[3] On deoxygenation state HbS results in formation of a gelatinous network of fibrous polymers called tactoids and thereby diminished solubility of the HbS, that stiffen and distort the RBCs producing a rigid, misshapen RBCs that traverse the small vessels with great difficulty or not at all leading to ischemia or infarction distal to the vessels and produce the vaso-occlusive crisis and early haemolysis due to decreased life span of RBC causing

chronic anaemia.[4,5] Despite substantial increased in knowledge in cellular, pathophysiological and physical basis of sickle phenomenon, there is disappointing lag in the application of this knowledge to designing of safe and effective form of therapy to prevent vaso-occlusive painful crisis and anaemia. Therapy directed for increase production of Foetal haemoglobin (HbF) by Hyroxyurea (HU), 5-Azacitidine and Decitabine is associated with risk of myelosuppression. Aspartame appears to have antisickling effects that prevents sickling and reverses sickling in reversible sickle cell (RS).[6] Therefore this study was undertaken to assess the efficacy of aspartame as prophylaxis to prevent anaemia and VOC.

The sickle polymers (tactoids) having diameter of 20 nm fibres and each consists of a helical stack of polymers with 14 strands, stabilized by hydrophobic bonding. The presence of hydrophobic side chain on valine substituted for a polar residue glutamic acid on the surface of the first (A) α- helix in the 3-dimentional structure of HbS creates an accessible apolar donor sticky site to a crevice (acceptor) between the E and F helix in the β-chain of an antiparallel HbS molecule in the acceptor site are phenylalanine 85 (phe 85) and the leucine (leu 88) from the F helix. Together the donor and acceptor in the reference β-chain interact with two other HbS molecules in the offset position to provide the major condensation events leading to double stranded helical stacks of indefinite length. As the polymer grows there is ample opportunity for the other free donor and acceptor sites on the surface of the strands to broaden the fibrils that distort the erythrocytes in to sickle shaped cells.[6]

Aspartame composed of two amino acids, L-aspartic-L-phenylalanine is a glucose analogue rapidly transported in to the RBC membrane transporter function sensitive to calcium ion concentration (inhibited by EDTA by Ca++ chelation). Aspartame can prevent fibril formation by competition and or interaction with or by getting inserted into the EF acceptor sites and prevent the α-helix that contain valine at β position from its delectation binding to the EF acceptor site and thus obstruct sickle polymerization.[6] The possibility of functional interruption may occur is appears to be the possible mechanism of action of aspartame. It is also speculated that the hydrophobic binding domain may able to accommodate the phenylalanine of aspartame whereas the trailing aspartic acid acts as a large hydrophilic extension that interfere with the approach of competing valine residue.[6] Aspartame's antisickling effect was first noted by Naguchi CT in 1982 and 1985.[7,8]

In 2001, Manion CV et al in their in-vitro study found that aspartame at 1mg/ml in whole blood of sickle cell disease patients decreased sickle cells formation significantly from 28% (control) to <14% and further decreased with 2 mg/ml to 12.08% at 2 hour. Aspartame also prevents sickling in presence of sickle promoting agents, i.e. Sodium Metabusulfite and Argon. Antisickling effect of aspartame was detectable for at least 27 hours.[6] In their in-vivo study of 23 subjects volunteers of 15 HbSS, 3 HbSβ^0Thal and 5 sickle cell trait (AS) a single dose of aspartame of 1.5, 3 and 6mg/kg body weight in subjects with HbSS and HbSβ^0Thal, sickle cell formation was decreased and maximal effect was seen with 6mg/kg body weight and peak effect lasted for more than 8 hours and persisted for 72 hours and the whole blood viscosity was decreased (but increased in AS cases).[6] Aspartame also has mild antithrombotic effect in human and mild antipyretic effect in animal study.[9]

METHODS

This was an open label, prospective, comparative single centre observational study conducted between November 2015 to April 2017 in the department of medicine VIMSAR, Burla, Odisha, (India). The primary objective was to evaluate the efficacy and safety of aspartame among patients with sickle cell disease to reduce anaemia and vaso-occlusive painful crisis. Consecutive HPLC diagnosed 208 cases of sickle cell disease (HbSS) with history of frequent vaso-occlusive crisis and/ or chronic anaemia after exclusion of phenylketonurea, vitamin B12, folic acid deficiency and recent blood transfusion were selected for the study from the outdoor and indoor department of medicine.

Patients were divided into study groups (n = 104) and control group (n = 104). In the study group 31 (30%) were on HU and 38 (37%) in control group were on HU (500mg/day). Baseline haemoglobin levels were measured in all cases and then monthly for six months. All patients in the study group were given a detail description of the study and their written informed consent was obtained prior to the study. Aspartame (sugar free Gold, 1 tablet = 18mg) was given to study group in the dose of 4mg/kg/day in three divided doses orally after approval of institutional ethical committee. All patients were followed with proper instructions for adherence to therapy and to notice any painful crisis or any adverse reactions in aspartame study group with telephonic communication and need for hospitalization. All patients were advised to attend the study centre monthly to measure haemoglobin level and to evaluate any adverse reactions. Data were collected and compared with control data by using SPSS software version 25.

RESULTS

Total 208 cases were studied after dropping out of 22 cases. In 104 cases of study group 64 (62%) were male and 40 (37%) were female and in control group 66 (63%) were male and 38 (37%) were female. Higher number of cases 62.5% (n = 65) in the study group were between age of 15 to 30 years followed by 34% (n = 35) were between 31-45 years of age. In the study group the mean baseline haemoglobin was 6.99 gm% (SD ±0.66) and after receiving aspartame it increased from 1st month significantly to a peak mean level of 8.43gm% (SD ±0.76) at the end of 3 months (P <0.05) and persisted

above baseline for entire six months of observation (P <0.05) (Table 1).

Table 1: Mean hemoglobin levels in patients.

Time	Mean	SD	P-value
Base line	6.99	0.78	
1st month	7.41	0.69	<0.05
2nd month	7.95	0.86	<0.05
3rd month	8.49	1.03	<0.05
4th month	8.17	0.89	<0.05
5th month	8.04	0.89	<0.05
6th month	7.98	0.87	<0.05

Table 1 shows the increase in mean haemoglobin levels in HbSS patients from mean baseline haemoglobin of 6.99 gm% to a highest mean of 8.49 gm% peaked at the third month of aspartame therapy (P <0.05).

Table 2: Mean hemoglobin levels in patients receiving aspartame.

	On Aspartame (n=104)		Control Group (n=104)		
Hb%	**Mean**	**SD**	**Mean**	**SD**	**P-value**
Base line	6.99	0.78	7.33	0.92	<0.05
1st month	7.41	0.69	7.12	0.81	<0.05
2nd month	7.95	0.86	7	0.8	<0.05
3rd month	8.49	1.03	6.98	0.76	<0.05
4th month	8.17	0.89	6.83	0.72	<0.05
5th month	8.04	0.89	6.7	0.76	<0.05
6th month	7.98	0.87	6.68	0.72	<0.05

Table 2 shows the mean Hb levels increased in each month on aspartame therapy and no rise of mean Hb levels in control group (P <0.05). There was no rise of mean haemoglobin level from baseline, rather decreased in control group (P <0.05) (Table 2).

In HU group add on aspartame had additional significant increase in haemoglobin level occurred as tested by unpaired 't' test, indicating aspartame is superior to HU. Table 3.

There was no significant mean haemoglobin level difference in different age and sex group as tested by ANOVA test, indicating that aspartame has equally effective in all age and sex group.

The incidence of vaso-occlusive painful crisis was very high (7.26%) in controlled group patients (P <0.05) by unpaired 't' test and 3.03% required hospitalization. The incidence of vaso-occlusive pain crisis was 3.58% in hydroxyurea patients and 1.28% required hospitalization. In aspartame study group 1.2% patients had milder types of vaso-occlusive crisis and only 0.3% required hospitalization. In HU+Aspartame 1.12% had vaso-occlusive painful crisis and 0.22% required hospitalization, indicate that HU has no additional benefit with aspartame (P >0.05) and aspartame prevent vaso-occlusive painful crisis and is non-inferior to HU.

Aspartame group had statistically significant increase in mean haemoglobin level than HU (P <0.05) (Figure 4) and prevent vaso-occlusive painful crisis better than HU.

Table 3: Mean hemoglobin levels in patients receiving hydroxyurea and aspartame in combination and monotherapy of hydroxyurea.

	HU + aspartame (n=31)		HU monotherapy (n=39)		
	Mean	**SD**	**Mean**	**SD**	**p-value**
Base line	6.73	0.69	7.17	0.85	<0.05
1st month	7.43	0.65	7.72	0.81	<0.05
2nd month	8.01	0.8	7.1	0.79	<0.05
3rd month	8.47	0.94	7.2	0.78	<0.05
4th month	8.19	0.73	7.07	0.76	<0.05
5th month	7.94	0.73	6.97	0.81	<0.05
6th month	7.91	0.79	6.88	0.72	<0.05

Table 3 shows the increase in mean Hb levels is more in patients on HU + aspartame as compared to HU alone. The increase in mean Hb levels is significant in patients on HU + Aspartame as compared to HU monotherapy as tested by unpaired t test.

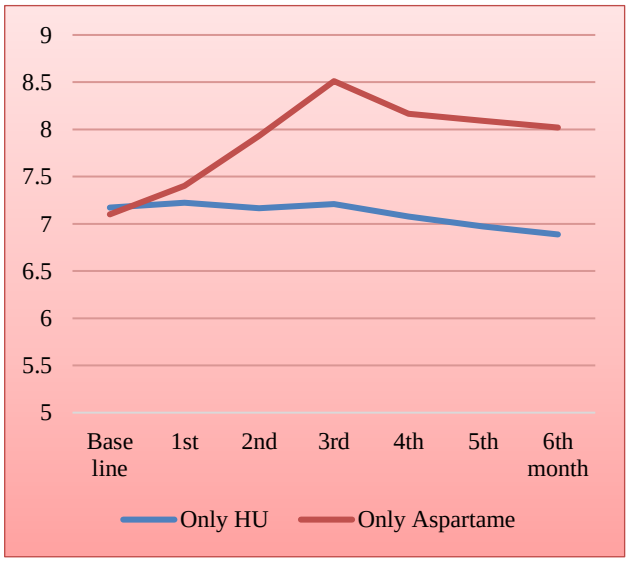

Figure 4: Mean hemoglobin levels in patients receiving monotherapy of aspartame and hydroxyurea.

Figure 4 shows the mean Hb levels were higher in patients on aspartame in comparison to HU. The increases in mean Hb levels were most prominent at the 3rd month of aspartame therapy.

DISCUSSION

The HbS fibre formation is time and oxygen concentration dependent and kinetics of HbS polymerization depends up on cell's degree of deoxygenation, with rapid deoxygenation multiple polymerization events results in a granular texture that does not alter the cell's shape, but when deoxygenation is slow or partially deoxygenated a single nucleus of deoxygenated HbS is formed and this nucleation is followed by growth and alignment of fibrils transforming the cells into a classic sickle shape.

Intracellular dehydration is caused by increased MCHC due to polymerization of HbS leading to red cell membrane damage and enhanced dehydration due to Potassium-Chloride (K^+-Cl^-) cotransport and the Ca^{++} activated K^+ efflux (Gardos Channel). The K^+-Cl^- cotransport channel is highly active in HbSS and HbSC red cell up on deoxygenation and cell swelling and acidification occurring at sites of stagnant circulation causing loss of Potassium and water and influx of calcium. The K^+-Cl^- channel can be inhibited by magnesium ion and the Gardos channel by Clotrimazole (Clo).[10,4]

The exact relation between intracellular polymerization of HbS and the protean clinical manifestation of the disease remains a mystery. Because the number of irreversible sickle cells (IRS) related to anaemia of the disease due to chronic haemolysis, but not explain the painful crisis and thus the reversible (RS) cells can cause vaso-occlusive crisis is suspected.[11] The RS cell have diminished cellular deformability and cause vascular obstruction at the precapillary sphincter level.[12] The RS cells have increased MCHC and higher viscosity.[13] There is complex interplay among sickle erythrocytes, endothelium, sub-endothelial matrix proteins, platelets, plasma clotting factors and certain inflammatory mediators.[10] Thus the causes of sickling may differ from event to event and its severity differ among patients. The duration and severity of pain crisis in sickle cell disease are notoriously variable and the natural course is one of spontaneous improvement.[5]

Any agent that alters these interactions and restore blood flow will be likely to beneficial in painful crisis and prevent anaemia. Pharmacologic induction of HbF formation by Hydroxyurea, 5-Azcytidine, and Decitabine are takes longer time with risk of myelosuppression and not suitable for acute painful crisis but can prevent or decrease the frequency and severity of painful crisis.[14,15] Aspartame appears to be effective in acute painful crisis and decreases severity of painful crisis acutely and duration of hospitalization.[16]

CONCLUSION

So far there are no reports of long-term clinical study of aspartame in sickle cell disease to prevent vaso-occlusive crisis and anaemia. It may be the first study of prophylaxis aspartame therapy in sickle cell disease. In this study aspartame therapy did not caused any adverse effects and appears to prevent both anaemia and painful vaso-occlusive crisis, most probably by reversing sickling of reversible sickle cell (RS) and preventing polymerization of HbS thereby increasing life span of RBCs. Aspartame is considered to be safe and effective with very large benefit to risk ratio and cost effective than HU.

It is as effective as HU and non-inferior to HU. As use of aspartame imposes no element of risk [Accepted Daily Intake (ADI)] of aspartame is 40 and 50mg/kg/day approved by EFSA and FDA respectively. Further prospective, multicentre, involving large number of patients, double blind, placebo control and dose finding studies are needed to confirm its efficacy in patients with sickle cell disease for the prevention anaemia and vaso-occlusive painful crisis and other protean manifestations.

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