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Effect of Aspartame on Hemoglobin in Sickle Cell Anemia

Tushar Kantee Behera[1], Jyoti Ranjan Behera[2], Anuradha Mishra[3], Subhasree Madhual[4]*

[1]Assistant Professor, Dept of General Medicine, SCB Medical College and Hospital, Cuttack, Odisha, India
[2]Associate Professor, Dept of Pediatrics, MKCG Medical College and Hospital, Berhampur, Ganjam, Odisha, India
[3]Associate Professor, Dept of Obstetrics and Gynaecology, MKCG Medical College and Hospital, Berhampur, Ganjam, Odisha, India
[4]Assistant Professor, Dept of Dermatology (Skin & VD), IMS and SUM Hospital, Bhubaneswar, Odisha, India

***Address for Correspondence:** Dr Subhasree Madhual, Assistant Professor, Dept of Dermatology (Skin & VD), IMS and SUM Hospital, Bhubaneswar, Odisha, India
E-mail: subhasreemadhual@gmail.com

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ABSTRACT

Background: Sickle cell disease (SCD) is a monogenic disorder characterized by aberrant hemoglobin S (Hb S) due to an A-to-T mutation in the β-globin gene. This leads to erythrocytes deforming or sickling, causing sickle cell crises, growth retardation, increased infection susceptibility, chronic hemolysis, multi-system organ damage, disability, and death. Anemia in SCD patients is primarily due to reduced red cell lifespan and hypersplenism in infants.

Methods: Patients from the VIMSAR Burla general medicine ward OPD/IPD were randomly assigned to a control group and a study group. Clinical examinations and histories were recorded, including hospital stay frequency, volatile organic compounds, blood transfusion history, and medication use. Patients on hydroxyurea continued their treatment. Conventional blood investigations (CBC, random blood sugar, liver function tests, serum urea, creatinine levels, and urine analysis) were conducted.

Results: The study showed that SCD patients in the study group taking 4 mg/day of aspartame had a significant increase in mean hemoglobin percentage starting from the first month and continuing until the third month, compared to the control group. The most substantial therapeutic benefit was observed in the third month.

Conclusion: Currently, hydroxyurea is the only FDA-approved effective treatment for SCD, but it it has several side effects. Aspartame, a safer over-the-counter medication, demonstrated greater efficacy than hydroxyurea in raising Hb% and reducing VOC, hospitalisations, and blood transfusions without adverse effects over a six-month trial. Aspartame is suggested as a potential first-line treatment for SCD, warranting a large-scale double-blind randomised controlled study to confirm its therapeutic benefits.

Key-words: Sickle Cell Disease, Aspartame, Hemoglobin, Hydroxyurea, Anemia, Red Blood Cells, Hypersplenism, Randomized Controlled Trial, Erythrocytes, Hemolysis

INTRODUCTION

Millions of individuals worldwide have SCD, a public health concern. Erythrocytes will physically distort or sickle because of the aberrant haemoglobin S (Hb S) produced by an A-to-T point mutation in the β-globin gene [1,2].

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This condition is monogenic. Sickle cell crises, growth retardation, increased susceptibility to infection, persistent hemolysis, consequences resulting in multi-systemic organ damage, disability, and mortality are typical presentations of this illness. Sickle cell crises and acute and chronic anaemia are the most significant clinical presentations. Reduction in red cell life span (T50Cr =8 days) is the primary cause of anaemia in SCD patients. Hypersplenism is a major source of RBC loss in young children with a substantially enlarged spleen [3].

Aplastic crisis and hemolytic process aggravation can lead to a significant decline in haemoglobin levels. Typically, the anaemia has normal MCV and MCH and is normochromic and normocytic [3,4]. The less well-

researched topic of anaemia in SCD is linked to long-term problems of the illness, including growth retardation, infections, peripheral vascular disease, transfusion-related complications, stroke, and so forth. Several research has focused on managing crises in SCD, but few have addressed anaemia in SCD [4,5]. The two amino acids alpha aspartic acid and L-phenyle alanine are combined to create aspartame, also known as L-Aspartyl L-Phynyle alanine. By penetrating the hydrophobic binding site, phenylalanine checks sickling prolongs the life of red blood cells and inhibits hemolysis. Sickle cell dropped from 28% to 14% *in vitro* research with aspartame and lowered at 2 mg/ml. [1] to cure SCD anaemia and prevent the need for blood transfusions [6-8].

Aspartame is an artificial sweetener sold over-the-counter (OTC) with almost no negative effects. This investigation aims to determine how aspartame affects haemoglobin levels in sickle cell disease. It is safe and doesn't negatively affect health [9]. Therefore, if it positively impacts lowering anaemia, it could be the most effective treatment for sickle cell disease.

MATERIALS AND METHODS

Research Design- This study was conducted on patients with SCD from the general medicine ward OPD/IPD of VIMSAR Burla. Participants were randomly selected and assigned to either a research or control group. A thorough history and clinical examination were conducted for all patients, documenting blood transfusion history, exposure to volatile organic compounds, frequency of hospitalizations, and medication use. Patients already on hydroxyurea were advised to continue their current treatment. Standard blood examinations included complete blood count (CBC), random blood sugar, serum urea and creatinine levels, liver function tests, and urine analysis. Baseline hemoglobin levels were measured for all patients. The trial group received aspartame at a daily dosage of 4 mg per kilogram of body weight, administered in three separate doses. Patients were instructed to follow up regularly and adhere to their treatment plan. Hemoglobin levels were estimated monthly for six consecutive months. Hospitalization requirements and VOC history were recorded. Patients requiring blood transfusions were excluded from the study. Out of an initial 230 participants, two died due to unrelated illnesses, four required blood transfusions and were excluded, and follow-up was not possible for 16 patients. This left 208 patients, with 104 in each study and control group. Data was collected, entered into a pre-defined format, and analyzed using SPSS software (version 25).

Inclusion Criteria

- Patients with HbSS, HbS beta thal, HbSC disease with Anaemia
- SCD with anemia with or without VOC.
- SCD patients with or without Hydroxyurea therapy.

Exclusion Criteria

- Sickle cell trait patient
- Patient with Phenylketonuria (PKU)
- Epilepsy
- Migraine
- Parkinsonism
- Pregnancy
- Severe Iron and megaloblastic anemia

Statistical analysis- The study used SPSS 25 for effective analysis. MS Excel was used to create graphs and other calculations. The continuous data, like hemoglobin percentage, were expressed as mean±standard deviation, while the discrete data were expressed as frequency and its respective percentage. The statistical analysis was done between the two groups. The study used ANOVA as the statistical tool for comparing the variables. The level of significance was $p<0.05$

Ethical Approval- The Study was approved by the ethical committee of VIMSAR Burla, Odisha, India.

RESULTS

Table 1 presents the age and sex distribution of the study group. The study group comprises 104 participants, with 64 males and 40 females. Among the age group 15-30 years, 41 males and 24 females totalled 65 participants. In the 31-45 age group, there are 20 males and 15 females, making up 35 participants. For the age group over 45 years, there are 3 males and 1 female, totalling 4 participants. This distribution shows that most of the study participants are younger, with a higher proportion of males across all age categories.

Table 1: Age and Sex Distribution in Study Group

Age group	Male	Female	Total
15-30	41	24	65
31-45	20	15	35
>45 yr	3	1	4
Total	64	40	104

Table 2 shows the change in mean Hb level in SCD patients after receiving aspartame with a mean Hb of 6.99 in baseline, which is increased each month with the highest mean of 8.49 in the 3rd month of aspartame therapy. p-value in each month is <0.05, which shows that the increased mean Hb level is significant throughout the study periods.

Table 2: Change in Mean Hemoglobin Level in Sickle Cell Disease Patients Receiving Aspartame

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 3 shows that the mean Hb level is increased each month in SCD patients on aspartame therapy and there is no rise in mean Hb level (rather decrease) in patients not on aspartame therapy. The p-value of <0.05 indicates the significant rise in Hb level after aspartame therapy compared to the control.

Table 3: Hb% In Aspartame Vs Non-Aspartame

	On Aspartame (n=104)		Not on Aspartame (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 4 shows that the baseline mean Hb level in patients on Hydroxyurea (HU) is higher than that in patients not on HU. However, there is no significant difference in Hb level in patients taking HU and aspartame compared to patients taking only aspartame. This indicates that HU has no additional effect on Hb level when given along with aspartame.

Table 4: Comparison of Hb% in SCD on Aspartame with HU vs Without HU

	Aspartame with HU (n=31)		Aspartame without HU (n=73)		
	Mean	SD	Mean	SD	p-value
Base Line	6.73	0.69	7.1	0.8	0.03
1st Month	7.43	0.65	7.4	0.72	0.798
2nd Month	8.01	0.8	7.93	0.88	0.662
3rd Month	8.47	0.94	8.51	1.07	0.649
4th Month	8.19	0.73	8.16	0.96	0.858
5th Month	7.94	0.73	8.08	0.95	0.854
6th Month	7.91	0.79	8.01	0.91	0.786

Table 5 shows that the increase in mean Hb level is higher in patients taking both HU and aspartame than those taking only HU. The increase in Hb level is significant in patients on both aspartame and HU compared to patients on only HU, as tested by unpaired t-tests.

Table 5: SCD On HU With Aspartame vs without Aspartame

	HU with aspartame (n=31)		HU without Aspartame (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 6 indicates no significant change in the mean hemoglobin (Hb) level after three and six months of aspartame therapy across different age groups, as assessed by the ANOVA test. This suggests that aspartame is equally effective in increasing Hb levels among all age groups. Specifically, the change in mean Hb percentage from baseline to the third month was 1.44 for patients aged 15-30 years, 1.69 for those aged 31-45 years, and 1.03 for those over 45 years. The changes were 0.9, 1.2, and 1.03 from baseline to the sixth month, respectively. The p-values for these changes were 0.57, 0.74, and 0.68, indicating no statistically significant differences between the age groups regarding Hb level changes.

Table 6: Change in mean Hb% in different age groups

	15-30yr (n=65)	**31-45yr (n=35)**	**>45yr (n=4)**
Change in Mean Hb% from baseline to 3rd month	1.44	1.69	1.03
Change in Mean Hb% from baseline to 6th month	0.9	1.2	1.03
p-value	0.57	0.74	0.68

DISCUSSION

The participants in the trial group were enrolled beginning in November 2015 and continued until April 2017. During this time, 230 patients were chosen, of whom 2 passed away, 4 were disqualified because they required blood transfusions, and 16 were lost to follow-up [10-12]. Thus, 208 patients in all—104 for each case and control group—were examined in this study. Of the 104 patients in the case group, 40 (38%) were female and 64 (62%) were male. Out of the 104 participants in the control group, 38 were female and 66 were male; the majority belonged to the 15–30 age range. After measuring the baseline haemoglobin levels in the case and control groups, it was discovered that the respective mean values were 6.99 and 7.33 [13-15]. After three months, the mean haemoglobin level was 8.49 mg/dl; after six months, it was 7.33 mg/dl.

The third-month mean Hb change from the baseline was determined to be significant (p<0.05). A substantial decrease in mean Hb was reported between baseline and six months (p-value<0.05). The mean Hb does not vary significantly between the third and sixth months (p>0.05) [16-18].

The greatest therapeutic benefit, or a rise in haemoglobin level, is shown at the end of the third month since there is no discernible change in mean haemoglobin between the third and sixth months [19-21]. This rise in haemoglobin levels will last until the completion of the six-month therapy period. When the mean haemoglobin level of aspartame-treated patients is compared to that of non-aspartame-treated patients, the aspartame-treated patients' mean haemoglobin level significantly increases after the first month [22]. Haemoglobin levels start to rise towards the end of the

first month and are found to be significantly higher. ($p<0.05$). Throughout therapy, patients using aspartame had a mean Hb level that was greater than that of patients not taking aspartame [23]. We compared the mean Hb level of patients on HU with patients not on HU among the patient's receiving aspartame. In both groups, the mean Hb level rose during the therapy. However, there are notable differences between the two groups. p-value is greater than 0.05. Thus, we conclude that HU offers no further benefit to SCD patients in the treatment of anaemia [24].

We examined the mean haemoglobin percentage of patients who took aspartame vs those who did not among the patients receiving HU [25]. After the first month, the patients in the aspartame-free group had greater mean and base haemoglobin. However, starting in the second month, the mean haemoglobin of patients taking aspartame with HU is noticeably greater than that of individuals getting HU alone ($p>0.05$). Evidence of aspartame's additional therapeutic benefit with HU. When compared, the difference in mean haemoglobin across age groups is insignificant ($p>0.05$). This demonstrates that aspartame's therapeutic efficacy is the same for all age groups [26].

CONCLUSIONS

The only FDA-approved treatment for SCD currently is Hydroxyurea therapy. Antimetabolite hydroxyurea has several side effects, including suppression of the bone marrow, an increased risk of subsequent cancer, teratogenicity, and decreased fertility owing to azospermia. Doctors are forced to utilise Hydroxyurea, an indirect antisickling drug, to avoid VOC and anaemia in patients with SCD because there isn't a substitute medication available. Aspartame, on the other hand, is an OTC sweetener that is safer, more affordable, and can prolong the life of red blood cells by inhibiting sickling. In our trial, it was proven to be more effective than Hydroxyurea in raising Hb%, avoiding VOC, lowering hospitalisation rates, and lowering the requirement for blood transfusions. No negative side effects were noted for the six months of the research. Our research suggests that aspartame may be used as a first-line treatment for sickle cell disease. A sizable double-blind, placebo-controlled, dose-finding randomised controlled study is necessary to determine the therapeutic benefit of aspartame in preventing anaemia and VOC in SCD.

CONTRIBUTION OF AUTHORS

Research concept- Anuradha Mishra, Subhasree Madhual
Research design- Tushar Kantee Behera, Anuradha Mishra
Supervision- Tushar Kantee Behera
Materials- Tushar Kantee Behera, Anuradha Mishra
Data collection- Tushar Kantee Behera, Anuradha Mishra
Data analysis and Interpretation- Jyoti Ranjan Behera
Literature search- Anuradha Mishra
Writing article- Tushar Kantee Behera, Anuradha Mishra
Critical review- Jyoti Ranjan Behera
Article editing- Jyoti Ranjan Behera
Final approval- Subhasree Madhual

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