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Impact of hydroxyurea on hospital stay & analgesic utilization in sickle cell anaemia with vaso-occlusive crises

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***Background & objectives*: Hydroxyurea (HU) has been useful in preventing sickle cell vaso-occlusive crises (VOC). A few studies also suggest utility of HU, during acute VOC. Sickle cell anaemia (SCA) is of high prevalence in western districts of Odisha State, India, and VOC is a common presentation, despite being mostly of Arab-Indian haplotype. This study was undertaken to evaluate the impact of HU on hospital stay and analgesic utilization in acute painful VOC of SCA.**

***Methods*: Homozygous sickle cell disease (HbSS) patients were categorized as cases who were receiving low-dose HU (10 mg/kg/day) and patients who were not on HU were considered as control. Days of hospital stay, analgesic utilization and visual analogue scale (VAS) score in patients were compared with that of control. Analgesics used to control pain were tramadol hydrochloride, ketorolac and diclofenac.**

***Results*: A total of 359 homozygous sickle cell disease (SCD) patients with VOC were studied (187 patients and 172 controls). The patient group had lesser mean days of hospital stay (1.4 days less than controls, $P<0.001$) and required lesser days of analgesic utilization than controls (1.18 days less than controls, $P<0.001$). Significant differences were observed between patients and controls concerning VAS score and amount of tramadol hydrochloride, ketorolac and diclofenac utilization ($P<0.05$).**

***Interpretation & conclusions*: In this study, HU was found to have beneficial effects in acute VOC of homozygous SCD, which includes shortening the duration of hospital stay and reducing the net amount of analgesic utilization during hospitalization.**

Key words Analgesics - hospital stay - hydroxyurea - sickle cell anaemia - vaso-occlusive crises

Sickle cell disease (SCD) refers to a group of symptomatic forms of sickle cell haemoglobinopathy which include homozygous sickle cell disease (HbSS), sickle cell anaemia, (SCA), and compound heterozygotes (HbSβ-thalassaemia, HbSC, $HbSD^{Punjab}$, HbSE disease, *etc*.) states. Molecular aggregates of sickle haemoglobin (HbS) damage the erythrocyte membrane and change the rheology of the erythrocyte in circulation, causing haemolytic anaemia, vaso-occlusion and vascular endothelial dysfunction[1].

Hydroxyurea (HU) is the first approved pharmacological therapy for SCD and is effective and safe both in children and adults[2-4]. The use of HU has decreased morbidity and improved survival in SCA. Sickle cell haemoglobinopathy is highly prevalent in the western districts of Odisha State of India (5-30%) and is a major public health problem of the State[5]. These patients manifest with severe clinical profile and are eligible for HU therapy. Studies from different parts of India found low dose of HU (10 mg/kg/day) to be effective and safe in reducing the frequency of vaso-occlusive crises (VOC) and the requirement of blood transfusion (BT) in patients with both homozygous SCA, $HbSD^{Punjab}$ and HbSβ-thalassaemia[6-8].

Pain of VOC is the major complaint of sickle cell patients throughout their lives. Use of HU prevents acute painful crises and acute chest syndrome (ACS) by modulating different factors involved in pathophysiologies like an increase in levels of HbF (fetal haemoglobin) and nitric oxide (NO), decrease in E selectin expression and reduction of neutrophils and platelets[9,10]. HU influences reduction of red blood cell (RBC) adhesion receptor expression and changes in endothelial cells, the degree of adherence of different blood cells, as well as modulation of levels of the determinants of intercellular and endothelial adhesion molecules such as soluble vascular adhesion molecule-1 (sVCAM-1) and myeloperoxidase. Decreasing sVCAM-1 and myeloperoxidase levels suggest a reduction in the erythrocyte–endothelial interactions and in neutrophil activity which may help to reduce the propagation phase of a VOC[11]. HU also causes reduction of hypercoagulability markers in SCD such as von Willebrand's factor and factor VIII and reduction of haemolysis induced NO production[12].

The effect of HU in controlling VOC is less studied than its relative role in populations of western countries. In India, there was a paucity of literature on the ameliorating effect of HU during VOC. In view of this, the present study was undertaken to assess the effect of HU on length of hospital stay and analgesic utilization during severe VOC in SCA patients of Indian origin.

Material & Methods

A prospective observational study was undertaken in the department of Internal Medicine, Veer Surendra Sai Institute of Medical Sciences and Research, Burla, Sambalpur, Odisha, India, during November 2016 to October 2018. Patients hospitalized in the department were considered for study after obtaining due approval from the Institutional Ethics Committee. Three hundred fifty nine patients of homozygous SCD with VOC between age 15 and 60 yr of both sexes, who satisfied inclusion and exclusion criteria and provided written informed consent were included in the study. The study population included 187 patients and 172 controls. Those who were on continuous HU therapy for at least 12 months and were continuing HU therapy during VOC were considered as cases. Those who had no HU therapy for at least three preceding months were considered as controls. The controls were of two categories; (*i*) who never came to the health facility for advising HU, and (*ii*) those who had discontinued HU on their own for more than three months. HU was not initiated during VOC in them and they were therefore considered as controls. Both groups received standard of care for sickle cell VOC. Detailed history, physical examination and treatment received were recorded for analyses. A power calculation was performed using software G*Power version 3.1.9.2[13]; a *post-hoc*, Wilcoxon-Mann-Whitney test between 187 patients and 170 controls patents provided a power of 99.58 per cent with two-tailed, normal distribution, effect size (ρ) of 0.5 and α=0.05 [type 1 error (false positive) probability of 5%].

Inclusion & exclusion criteria: Patients having homozygous SCD (HbSS, SCA) with VOC between ages 15 and 60 yr were included in the study. The patients having following characteristics were excluded from the study groups: (*i*) pregnancy, (*ii*) lactation, (*iii*) moderate to severe renal impairment, (*iv*) active hepatic disease, (*v*) blood haemoglobin <5 gm/dl, (*vi*) absolute neutrophil count (ANC) <2 x 10^3/µl, and (*vii*) platelet count <80 x 10^3/µl.

Laboratory investigation:

Collection of sample: Five millilitres (ml) of blood was collected from each patient during the hospital stay, of which 3 ml was transferred to EDTA containing vacutainer for complete blood count (CBC), sickling slide test, Hb electrophoresis (*p*H 8.6) and cation exchange high performance liquid chromatogram (CE-HPLC). The rest 2 ml of blood was transferred into serum clot activator vacutainer for estimation of biochemical parameters. Subsequently, 3 ml of blood was taken daily for CBC and biochemical estimation during follow up.

Biochemical and haematological investigations: Biochemical parameters, such as total

serum bilirubin, direct and indirect (BIL-T, BIL-D and Bil-I) bilirubin, serum creatinine (Cr), serum urea and lactate dehydrogenase (LDH) were studied using Cobas Integra 400 Plus (Roche Diagnostics Ltd., Rotkreuz, Switzerland), as per the manufacturer's instructions and standard protocols. The CBC was performed using Sysmex KX 21 (Sysmex Corporation, Kobe, Japan); CBC not only helped in excluding patients when counts were low but also to find if one would have a microcytic hypochromic blood picture suggestive of co-existent β-thalassaemia when HbA_2 in CE-HPLC was found to be between 3.5 and four per cent.

Sickle cell anaemia (SCA) diagnosis: Diagnosis of SCA was made by sickle slide test, alkaline agarose gel haemoglobin electrophoresis (*p*H 8.6) and CE-HPLC done sequentially. Patients found positive for sickled RBC in slide test and S or SF band with or without any other variant on Hb electrophoresis in absence of blood transfusion (BT) within the last three months were diagnosed as having SCD. CE-HPLC was done for patients with SCD to quantify different haemoglobin fractions by using Variant II, β-thalassaemia short program (Bio-Rad Laboratories, Hercules, CA, USA). Homozygous SCD (HbSS, SCA) was diagnosed when CE-HPLC showed HbS (75-95%), HbF (1-20%) and HbA2 (2-3.5%). In case of patients with CE-HPLC showing HbS (>50%), HbF (1-20%) and HbA2 (4-10%) with CBC showing microcytic-hypochromic blood picture were diagnosed as HbS/beta-thalassaemia.

Vaso-occlusive crises (VOCs): It was defined as an acute painful event affecting bones of extremities, rib cage, vertebra and pelvis that required oral or injectable analgesics and that lasted for 4 h or more when no other cause could explain the symptom[14].

Standard of care of VOCs: Analgesics were started within half an hour of hospitalization. The opioid analgesic used was tramadol hydrochloride (tramadol HCL) due to the non-availability of morphine. Ketorolac or diclofenac injections were given as add on for the relief of pain. An oral or intravenous fluid (0.9% NaCl or 5% dextrose solution) was used for maintaining hydration. Antibiotics used were ceftriaxone injection with or without oral macrolides like azithromycin and others in case of infection. Oxygen was supplemented in case of oxygen saturation falling below 95 per cent and simple transfusion was given in case of severe VOC[1].

Protocol for patients and controls:

Treatment and monitoring of patients: All SCA patients with VOC who fulfilled the inclusion and exclusion criteria were allowed to continue low fixed-dose HU therapy (10 mg/kg/day). In addition, they received tramadol HCL intramuscular injections 300 mg/day in three divided doses. If pain persisted during monitoring of pain, diclofenac or ketorolac intramuscular injections were added to control pain effectively, the maximum dose of injection diclofenac used was 150 mg/day given in divided doses and the maximum dose of ketorolac used was 90 mg/day in divided doses[15]. In addition to this, fluid, antibiotics, oxygen and BT were administrated as per the standard of care for VOC patients mentioned above. As the pain subsided the analgesics were tapered one by one. Assessment of pain intensity in SCA with VOC patients was evaluated at admission and then at 24 h intervals until discharge using a modified visual analogue scale (VAS)[16]. The amount of analgesics used and duration of stay in the hospital was recorded. Monitoring of adverse effects of HU was done by daily estimates of haemoglobin (Hb%), differential count (DC), total leukocyte count (TLC), ANC, and total platelet count (TPC). Liver function test (LFT), blood urea and serum creatinine were estimated for monitoring complications on alternate days; LDH was estimated at admission and at discharge as maker of haemolysis.

Patients were asked to rate pain intensity by placing a mark on a 10 point modified VAS[17,18]. The VAS was horizontally positioned with the extremes labeled 'no pain' and 'worst possible pain'. One minute later, patients were asked to rate their pain severity again on a fresh VAS without reference to the first measurement. A minute was chosen as the time interval between paired ratings under the assumption that most pain would not change within one minute. Then according to profiles of VAS rating, the score was noted daily and analgesics were adjusted accordingly.

Treatment and monitoring of controls: Controls were treated as per standard of care with analgesics and others as mentioned in the patient group. The controls were observed for the duration of stay in the hospital, amount of analgesic used and pain intensity experienced on day-to-day basis as in patients using the modified VAS at admission and then at 24 h interval daily till discharge. Daily estimation of Hb per cent, DC, TLC,

ANC, TPC and alternate day estimation of LFT, blood urea and serum creatinine were carried out in controls for comparison. Serum LDH was estimated in controls at admission and discharge as a marker of haemolysis.

Statistical analysis: Data were collected systematically in pre-designed format and statistical analyses were conducted using GraphPad Prism Version 5.00 (GraphPad Software for Windows, San Diego, CA, USA) and by SPSS v23.0 (Statistical Package for Social Sciences for Windows, IBM Inc., Chicago, IL, USA). Analyses as per gender were done by Fisher's exact test, whereas age, hospital stay and analgesic utilization between cases and control were compared using Mann-Whitney U test. The differences between admission and discharge of patients and controls were analyzed by Tukey-Kramer multiple comparison test. One-way repeated measures analysis of variance (ANOVA), and Wilk's Lambda multivariate test were conducted to compare the hospital stay on each analgesic consumed among patients and controls.

Results

A total of 359 SCA (HbSS only) patients (n=187) with VOC and controls (n=172), satisfying the selection criteria, were included. The number of male and female among patient group were 129 (68.98%) and 58 (31.01%), and in the control group were 107 (62.20%) and 65 (37.79%), respectively. There was no significant difference (P=0.18) in gender distribution among the groups. The mean age (±SD) of patients and control were of 27.10±11.29 and 24.71±7.82 yr, respectively. There was no significant difference (P=0.27) in the age distribution between the groups (Table I).

Duration of hospital stay and analgesic utilization (days): Among the patients and controls with SCA, it was found that controls had significantly (P<0.001) longer duration of hospital stay than cases (6.55±1.7 *vs*. 5.16±1.29 days). In addition to this, control group had a longer duration of analgesic utilization needed for recovery from VOC (5.98±0.97 days) compared to patients (4.8±0.89 days), which was found to be significant (P<0.001) (Table I). There was also significant difference in the number of VOCs experienced by patients and controls in past; the patients suffered a lesser number of VOCs than controls. Since this study was mainly intended to capture changes during the present hospital stay, the number of VOCs suffered previously was not taken into account. The patients after recovery were advised to continue HU treatment and the controls after recovery were also advised for initiation of HU therapy. Adherence to such practices was beyond the scope of this study.

Table I. Gender, age distribution, duration of hospital stay and analgesic utilization

Variables	Cases (n=187)	Controls (n=172)
Gender distribution		
Male	129	107
Female	58	65
Age (yr)[†]	27.10±11.29	24.71±7.82
Hospital stay (days)[†]	5.16±1.29***	6.55±1.7
Analgesic utilization (days)[†]	4.8±0.89***	5.98±0.97

P ***<0.001 compared to controls. [†]data presented as mean±SD

Analgesic utilization: The mean amounts of cumulative analgesic used in patients for tramadol HCL, diclofenac and ketorolac were 1215.5±250, 405±80.15 and 188.03±54.26 mg, respectively; whereas, in controls mean dose were 1493.02±257.42, 459.21±128.4 and 211.64±60.23 mg, respectively.

The daily doses of analgesics of patients and controls were compared which showed that there was a significant relation between hospital stay (day) and analgesic utilization among patients and controls. The study patient group used less amount of analgesics than controls; tramadol HCL, Wilk's Lambda=0.729, F(9, 349)=14.401, P<0.001; and for usage of additional NSAIDs like diclofenac, Wilk's Lambda=0.964, F(6, 352)=2.205, P=0.042; ketorolac, Wilk's Lambda=0.931, F(6, 352)=4.346, P<0.001 (Table II).

Pain intensity: The intensity of pain in patient group was comparatively less at admission (by VAS) having a mean score of 7.23±1.13 as compared to the control score (7.97±0.86). The average pain score also showed a significant difference until six days from the day of admission (P<0.001). None of the patients experienced pain after eight days whereas, in the control group pain persisted in a few up to 10 days. The left shift line of patient group in Figure indicates that there was a quick and early improvement of patients than controls. There was a significant difference in the pattern of VAS between patients and controls (Wilk's Lambda=0.67, F[9, 349]=19.136, P<0.001), which indicated that HU played a key role in decreasing pain

Table II. Wilks' Lambda multivariate test results of hospital stay (in days) and analgesic utilization in cases and controls

Variables	Wilks' Lambda multivariate tests				
	Value	*F*	Hypothesis df	Error df	*P*
Tramadol HCL (mg)	0.729	14.401	9	349	<0.001
Diclofenac (mg)	0.964	2.205	6	352	0.042
Ketorolac (mg)	0.931	4.346	6	352	<0.001
VAS (cm)	0.67	19.136	9	349	<0.001

Df, degree of freedom; F, one-way ANOVA; VAS, visual analogue scale; HCL, hydrochloride

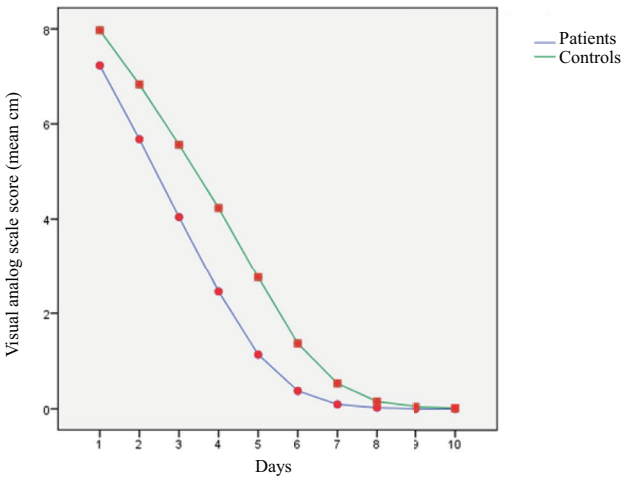

Figure. Daily assessment of pain intensity in patients of sickle cell anaemia with vaso-occlusive crises.

intensity in patients with SCA in the studied population (Table II and Figure).

Effect on haemolysis: The effect of HU on haemolysis was assessed by estimation of haemoglobin level and serum LDH level as well as serum bilirubin level. The haemoglobin level in patients at admission was 8.71±0.98 g/dl and at discharge was 8.87±1.37 g/dl and that of controls was 6.62±2.99 g/dl at admission and 7.83±0.98 g/dl at discharge, respectively (P<0.001). Serum LDH level at admission was 837.47±298.91 U/L in patients on HU and 1309.61±971 U/L in controls. Similarly, at discharge serum LDH levels in patients and controls were 800.05±293 U/L and 1257±983.3 U/L, respectively (Table III); controls were having a significantly higher level of serum LDH both at admission and at discharge (P<0.001) compared to patients.

Serum bilirubin level in SCA with VOC: The values of mean Bil-T, Bil-D and Bil-I levels were estimated at admission, and discharge in both patients and controls (depicted in Table III). The differences between patients and controls at admission and discharge were not found significant (P>0.05).

In our study, eight (4.65%) out of 187 patient, and 15 (8.72%) out of 172 controls developed ACS. There was no significant difference between the two groups (P=0.13) in this regard.

Adverse effects on haematological and biochemical parameters: Monitoring of myelotoxicity during acute crises was carried out by periodic estimation of Hb, TLC, TPC, ANC and renal functions were assessed by blood, serum urea and creatinine level. None of the patients had evidence of leucopenia, neutropenia and thrombocytopaenia.

Discussion

Indian SCA patients have been symptomatic, despite having a higher level of HbF; the most common complication is VOC, which needs immediate medical care or else this may lead to the death of the patients[19,20]. In the present study, among male and female patients with SCA in both patient and control groups, numbers of male patients were more, which could be due to differential care seeking in this population. There was no significant difference in the mean age of patients and controls (Table I). However, it was observed that most VOC occured during the third decade of life which was similar to the study by Dampier *et al*[19].

It was observed that patients recovered earlier than controls. Those, on HU therapy, started recovering from the third day but non-HU patients took a minimum of four days for resolution of VOC. The mean duration of hospital stay, in patients, was nearly 1.4 days lesser than that in controls, which is in contrast with the multicenter study of HU of two days[21]. This difference in recovery patterns could be due to our patients having a higher HbF level.

Table III. Haematological and biochemical parameters of patients and controls during admission and discharges

Parameter	Admission (A)			Discharge (B)			*P*	
	Case (C)	Control (D)	*P*	Case (C)	Control (D)	*P*	AC versus BC	AD versus BD
Hb (g/dl)	8.71±0.98	6.62±2.99	<0.001	8.87±1.37	7.83±0.98	<0.001	NS	<0.001
TLC ($^{x}10^{3}$/µl)	10.92±1.07	12.03±6.31	NS	10.39±3.85	10.21±3.42	NS	<0.001	<0.001
TPC ($^{x}10^{9}$/µl)	2.39±1.07	1.69±0.92	<0.001	2.33±0.65	1.88±0.75	<0.001	<0.001	NS
ANC (number/µl)	7181.67±3503	8755.59±5720.1	<0.001	6644.86±2732.2	7166.81±3085.6	NS	<0.001	NS
Creatinine (mg/dl)	0.8±0.22	1.18±0.4	NS	0.78±0.14	1.07±0.5	NS	NS	NS
Urea (mg/dl)	21.89±8.3	42.89±27.92	<0.001	21.76±5.4	30±12.69	<0.001	NS	NS
S.LDH (U/l)	837.47±298.91	1309.61±971	<0.001	800.05±293	1257±983.3	<0.001	NS	NS
Bil-T (mg/dl)	3.0±2.0	4.97±3.5	NS	2.12±0.86	4.4±4.3	NS	NS	NS
Bil-D (mg/dl)	0.88±0.76	2.77±2.6	NS	0.73±0.39	2.6±3.3	NS	NS	NS
Bil-I (mg/dl)	2.12±1.3	2.19±1.44	NS	1.39±0.52	1.75±1.12	NS	NS	NS

Hb, haemoglobin; TLC, total leucocyte count; TPC, total platelet count; ANC, absolute neutrophil count; S.LDH, serum lactate dehydrogenase; Bil-T, serum total bilirubin; Bil-D, serum direct bilirubin; Bil-I, serum indirect bilirubin; NS, not significant

In the current study, the minimum duration of analgesic utilization was three days in case of patients and four days in case of controls. While the maximum duration was seven and ten days in patients and controls, respectively. Most of the patients (49.73%) used analgesics up to five days compared to similar proportion of controls (48.84%), who used these for six days. From this observation, it is indicated that HU decreases analgesic utilization duration by one day. This finding of reduction in analgesic utilization in ambulatory SCD with painful crises is corroborated by the study of Smith *et al*[22], who had similar observation.

Due to regulatory prohibition and the non-availability of morphine in our setup, tramadol HCL was used as an alternative. The present study showed that HU patients group required tramadol for significantly lesser number of days with lesser cumulative dose as compared to the controls. The usage of additional NSAIDs (diclofenac and ketorolac) showed a significant difference as well. A similar finding was reported by Ballas *et al*[21], highlighting that HU reduced the net amount of opioids utilization.

Patients on HU had significantly less pain intensity (VAS score) at admission compared to controls. Present study was the first to measure VAS score in patients of sickle cell VOC with and without HU therapy during hospital stay.

The haemoglobin level in patients at admission was higher as compared to controls, which could be due to the effect of HU therapy in SCA patients[7]. This beneficial effect of HU on haemoglobin level persisted during VOC till discharge and maintained at a higher level compared to controls probably by reducing haemolysis.

Haemolysis measured by estimation of serum LDH in an earlier study from our center observed that there was an increasing trend of LDH level observed from milder to severe VOC in SCA patients[23]. Many other studies support this finding[24,25]. In the present study, it was found that those on HU had lower serum LDH levels than non-HU patient group at both admission and discharge. Lower LDH levels in cases suggest that HU might have some protective effect against haemolysis during VOC. Serum bilirubin level in the control group was higher; although not to a significant extent as compared to patients, which could be due to a higher degree of haemolysis in them.

In a previous study, a decreasing trend of platelet count from mild to severe VOC was noted[23]. In the present study, TPC was lower in controls as compared to patients at both admission and discharge. This could be due to more platelet getting stuck in the lumen of small blood vessels in controls than in cases, leading to more pain intensity as marked by VAS score. However, thrombocytopaenia was not found in any of the patients

or controls. ANC was lower in patients treated with HU compared to non-HU patients at both admission and discharge. This could be due to the myelosuppressive effect of HU[26]. On myelotoxicity monitoring during acute crises in patients, it was observed that none of them had evidence of neutropenia and thrombocytopenia, which was in contrast with the published findings as reported by Wong *et al*[26], probably due to the low dose of HU used in our study.

Blood urea and serum creatinine were monitored at admission and discharge for any renal toxicity on account of the use of NSAIDs as well as possible renal dysfunction due to VOC. Although, mean urea level was lower in patients than controls at admission and discharge, the levels were within normal range except for a mild rise found in controls at admission. Serum creatinine levels in patients and controls were within the normal range as well; no renal dysfunction was obvious. Reports highlight that a low dose of HU may have beneficial effect in SCA patients by preventing microalbuminuria and subsequently renal dysfunction[27]. However, this aspect was not evaluated in the present study.

As per an earlier study[28], it could be stated that our study population was mostly from Arab-Indian haplotype because we studied the same population. The strength of our study was a thorough evaluation of data although lack of masking was a limitation.

This study showed the beneficial effects of HU in acute VOC of SCD, which included shortening the duration of hospital stay and duration of analgesic utilization as well as reduction of the net amount of analgesic required during hospitalization. HU also reduced the intensity of pain during VOC. HU utilization during VOC was associated with less severe anaemia and lower serum LDH suggesting HU's protective effect against haemolysis. The role of HU in preventing the progression of VOC to ACS still remains unclear; a larger number of patients may be studied to test this hypothesis. There were no serious adverse effects observed in the current study of low dose HU utilization in SCD with acute painful VOC.

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***Conflicts of Interest*:** None.

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