Effect of Hydroxyurea on β-Thalassaemia major & Hb E/β Thalassaemia .

Presented
By

Prof. (Dr.) Mohiuddin Ahmed Khan.
Professor & Head , Deptt. of Haematology.
Dhaka Medical College & Hospital Dhaka. .

Introduction

- Thalasaemia is the most common inherited genetic disorder.
- Present situation: in world wide
- 6.5% of world populations are carriers of different inherited disorder of Hb.
- >100 millions β-thalassaemia carrier.
- About 100,000 children with Thalassaemia major are born each year.
- Hb-E carriers are 53 millions

- Present situation in Bangladesh: No definite data regarding carrier status is exist.
- According to WHO reports; 4% Hb-E carrier & 3% are β-Thalassaemia carrier.
- 4.8 millions carrier of Hb-E & 3.6 millions carriers of β-Thalassaemia.
- Affected birth per thousand of Hb-E & β-Thalassaemia are 0.3 & 0.1 respectively.

Present treatment options:

- Blood Transfusion:
- Iron chelation therapy:
- Bone marrow transplantation:

New approach :

Drug treatment to reduce or eliminate need of transfusion, thereby no transfusion related complications.

- Drugs are: HU, 5-azacytidine, Ara-C, EP, Sodium-butyrate.

Mechanism of action:

- An increase in Y chain production which affect globin chain imbalance & enhance Hb-F synthesis, rendering erythropoisis more efficient and prolonging erythrocyte survival.
- Act by promoting the transcription of Y-mRNA molecule or
- By recruiting a population of erythroid progenitors in which Y to β-chain synthesis switch has not been turned off completely.

Aim & Objectives

- To observe the efficacy of HU on Hb-E/ β thalassaemia & β-thalassaemia major.
- Stop or reduction of blood transfusion, thereby no iron overloading complications
- To observe tolerance & toxicity of HU.

Materials & Methods

- **Study design:**

  This was a prospective case controlled study on thalassaemia.

- **Study population:**

  Group-I : Test subjects. 33 transfusion dependent thalassaemia patients (E/β = 26 + T. major = 07).

  Group-II : Control cases. 30 transfusion dependent thalassaemia patients (E/β = 24 + T. major = 06).

- **Study period:**

  One year (Oct, 2000 to Sept, 2001).

- **Case selection:**

  By following diagnostic, inclusion & exclusion criteria.

- **Diagnostic criteria:**

  The cases were diagnosed on the basis of clinical history, physical examination, Lab. test (CBC, PBF) & confirmed by Hb-electrophoresis.

- **Inclusion criteria:**

1. Transfusion dependent E/ β thalassaemia & thalassaemia major.
2. Age more than 5 years.
3. Patients of both sexes.
4. Normal LFT & RFT.

- **Exclusion criteria:**

1. Age < 5 yrs.
2. Abnormal LFT & RFT.
3. Associated with complications e.g; heart failure, hypersplenism, severe general illness.
4. Failure to complied with scheduled medication.
5. Other systemic illness.

Study plan:

1. The benefit & hazards of HU were discussed to patients & parents and informed consent were taken.
2. A standard questionnaire was supplied
3. Clinical examination done
4. Investigations: CBC (by autoanalizer), Reticulocyte count, Hb-electrophoresis (by cellulose acetate at alkaline PH), PBF, Hb-F estimation , S.ferrin , LFT, RFT done as baseline tests.

Hydroxyurea administration:

- HU was used in escalating dose ranging from 10 -18 mg/kg/day for one year.
- 4 wks interval dose were increase by 3-5mg/kg/day and confined in that dose if no toxicity developed.
- In case of young children total weekly dose calculated & given 3-4 divided dose per week.
- In pre-toxic or toxic condition; HU dose reduced or withhold temporarily.

Pre-toxic: ANC=<$3x10^9$/L, ARC=<$125x10^9$/L, Hb=fall>15%, PC=$125x10^9$/L

Moderately severe Toxic: ANC= <$2x10^9$/L, ARC= $80x10^9$/L,
Hb= fall>20%(<6g/dl).

- **Placebo administration:**

  All the control cases (group-II) were given vitamin-B complex capsule everyday.

- **Other management:**

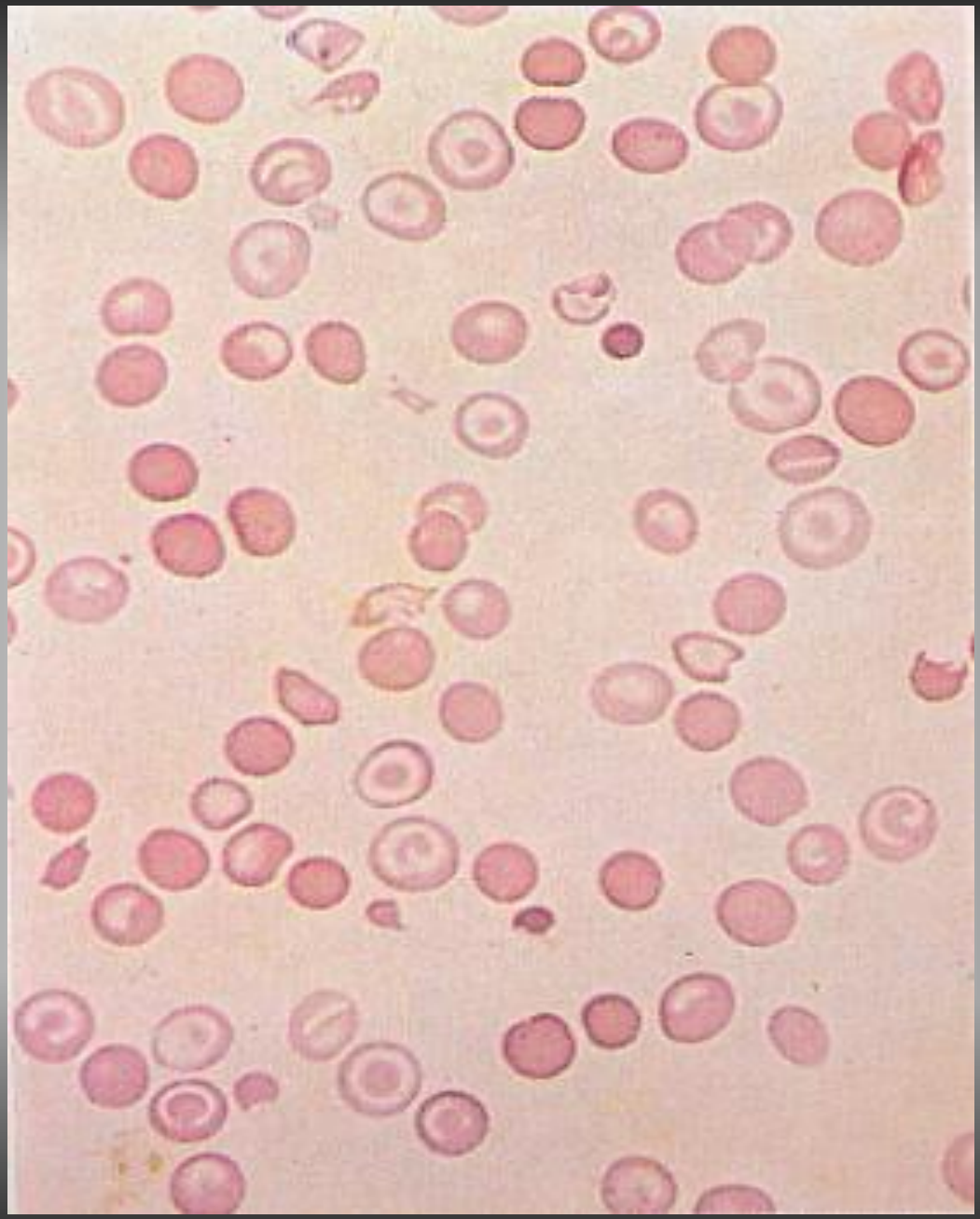
  i. Blood transfusion when indicated.
  ii. Folic acid orally (5mg twice weekly).
  iii. Dietary advice.

- **Follow up:**

  Patients were followed up initially monthly for first 3 months, then every 3 monthly up to 1 year at the study centre.

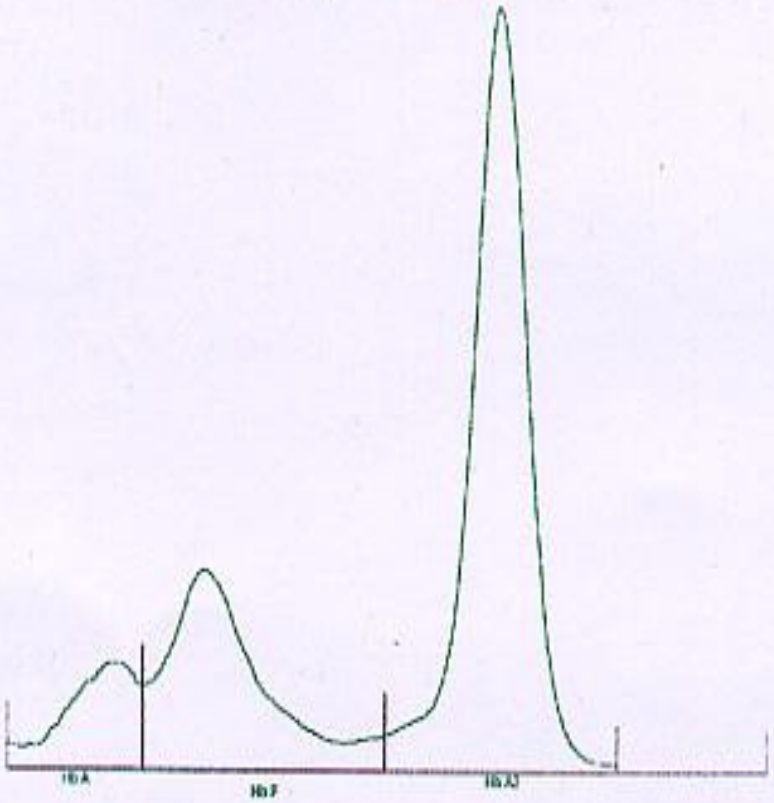
- **Follow up schedule included:**

  1. Physical examination.
  2. Drug compliance.
  3. Investigation: CBC, PBF, LFT, RFT, Hb-F (estimation three monthly).
  4. A follow up sheet was maintained for each patient.



Hemoglobin Electrophoresis

On agarose gel (Hydragel)



Conc of Hb in g/dl: 4.4

Fraction	% of Hb	g/dl	Normal Value(%)
Hb A	10.9	0.5	96.5 - 100
Hb F	26.9	1.2	0.0 - 2.0
Hb A2	62.2	2.7	1.0 - 3.5

RESULTS

Table-1: Distribution of patients by type of thalassaemia.

Type of thalassaemia	**Group-I (S)**	**Group-II (C)**	Total
β-thalassaemia major	07 (21.2%)	06 (20%)	11
Hb-E/ β thalassaemia	26 (78%)	24 (80%)	42
Total	**33**	**30**	**63**

Table-2: Distribution of patients by age.

Age	Group-I (S)	Group-II (C)	Total
5-10 yrs.	19 (57%)	18 (60%)	**37 (59%)**
11-15 yrs.	10 (31%)	08 (27%)	**18 (28%)**
16-20 yrs	04 (12%)	04 (13%)	**08 (13%)**
Total	**33 (100%)**	**30 (100%)**	**63 (100%)**

Table-3: Clinical findings: N=33 (study group)

Clinical finding	Present	Absent
Anaemia	33 (100%)	00
Jaundice	27 (81.8%)	06 (18.2%)
Splenomegaly	10 (30.3%)	00
Hepato-spenomegaly	16 (48.5%)	04 (12.2%)
Spelectomy done	03 (9%)	00
Facial bony changes	03 (9%)	30 (91%)
Dark skin	02 (6%)	31 (94%)

Table 4: Haematological response to HU in 32 subjects after completion of 1 yrs. treatment.

Subject	Hb=	Gm/dl	Hb-F	in %	MCV	(fl)	MCH	(pg)	RC	in %	Bilirubin	mg/dl
No.	A	B	A	B	A	B	A	B	A	B	A	B
1	9.1	9.0	29	30	62	63	21	22.5	6.5	6	1.9	1.8
2	6.0	8.1	38	56	65	77	18	23	10	5	4.9	3.1
3	7.8	7.7	35	34	61	62	20	20	8	8.5	2.1	2
4	7.0	9.1	41	58	64	80	19.5	25.5	8.5	4.5	3	1.8
5	6.0	6.1	51	50	58	60	18.5	18.0	7.5	7.5	3.6	3.5
6	7.5	7.6	48	47	64	65	21	21.5	8	8	2.5	2.5
7	7.4	9.6	43	61	68	82	20	26	9	5.5	1.9	1.3
8	7.4	7.0	45	44	67	66	20	19.5	8	8.5	2.1	2
9	6.8	6.6	49	48	61	60	21	21	9.5	9	2.3	2.2
10	7.9	10.1	50	67	70	87	20.5	27	7.5	4	1.8	1
11	5.4	6.8	35	50	65	76	19	22	10	6	3.5	2.6
12	7.8	7.9	27	28	60	61.5	20	21.5	8.5	8	1.9	1.9
13	6.0	5.8	36	36.6	58	60	14.1	15.8	9.5	9	1.8	1.7
14	6.4	6.5	52	56	58	61	19.5	20.5	12	11.5	1.9	1.8
15	7.8	9.1	44	63	64	80	18	25	9.5	4.5	4.2	2.8

16	7.0	7.1	71.5	72	61	62	21.5	22	8.5	8	1.7	1.8
17	8.2	8.1	58.3	58	68	67	22.5	22	8	8	1.8	1.8
18	7.7	7.8	35	37	63	65	19.7	19.9	8	8	2.8	2.7
19	7.3	8.8	25	40	70	86	22	25	9	3.5	1.9	1.1
20	8.7	8.9	36	38	71	72	22	24	7.5	7	1.5	1.4
21	7.1	7.5	77	79	65	67	20.5	21	9.5	8.5	1.8	1.7
22	6.1	6.2	48	49	69	70	20.9	21.1	8.5	8	2.3	2.2
23	8.0	9.8	56.5	64	68	86	20.5	25.5	8	4	2.9	2
24	7.3	7.4	50	48.5	67	68	21.6	21.9	9	8.5	3.3	3.2
25	5.5	7.0	17.8	30	52	62	14.9	20	12	6	2.7	1.8
26	7.5	7.7	77	78	59	62	20.9	21	10	9	3.6	3.5
27	5.5	6.5	46	49	57	63	17.5	19.5	9.5	8	3.3	3
28	5.1	5.3	67	68.5	64	66	21	22	10	9	3.6	3.5
29	6.6	8.5	75	91	70	81	21	27	9	4	3	1.7
30	8.0	9.4	41.5	57	68	83	21	26	7	3.3	1.8	1.3
31	8.0	8.1	58.9	58.5	63	64	18.4	19	8	8	2.5	2.4
32	7.4	9.5	54	75	65	85	20	27	8.5	3.5	2.2	1.5

A: Prior to HU therapy B: value after 1 yrs completion of HU

Yellow rows shown significant responses

Table 5: Haematological response to placebo therapy in 30 control cases after 1 yr.of treatment.

Subject	Hb=	Gm/dl	Hb-F	in %	MCV	(fl)	MCH	(pg)	RC	in %	Bilirubin	mg/dl
No.	A	B	A	B	A	B	A	B	A	B	A	B
1	6.1	6.1	49	47.2	64	65	20.5	21	8.5	8.5	3.2	3.1
2	6	5.8	83	82	58	57	19.3	22	7.5	7.5	3.5	3.3
3	5	5.1	51.5	50	56	60	18.6	18.3	8	7.5	1.9	2
4	9	9.5	50	51.4	70	72	21	21.5	7	7	1.8	1.7
5	7	7.1	43.5	45	68	64	20	20.5	7.5	7	2	2.1
6	7.5	7.8	36	34.2	61	63	21	22	9	9.5	1.6	1.5
7	8.5	8	48.8	39	69	56	22	21.5	7	8	2.8	2.6
8	9	8.8	76	78	69	67	22.5	23	8.5	8	2.4	2.4
9	6.4	6.7	83	50.4	55	59	20	19	10	10.5	3	2.8
10	7	6.6	39	41	66	61	20.5	21.7	9	8	2.5	3.2
11	5.5	4.8	56.2	55	56	59	18.7	19	10.5	9.5	3.3	3.1
12	9	9.1	28	30	69	70	22	22.5	6.5	5.5	1.8	1.6
13	7	7.2	37.3	39	71	72	23	22.3	9	8	2.1	2.3
14	6.9	6	28	27.4	66	60	18.3	18	7.5	8	2	2
15	5.4	6	78	74	57	59	18	18.7	11	9.5	3.3	3

16	8.3	8.5	75.3	77	60	60	21.3	22	9	8	1.5	1.1
17	5.2	5.9	42.4	45	58	60	19.6	20.1	11.5	10	2.4	2.2
18	5.8	5.5	34	32	59	60	20	20.8	7.5	7	2.4	2.3
19	6.8	6	30	28.5	64	60	18.9	18.7	12	9.5	2.3	2.5
20	6	7.7	47.5	49.3	58	60	19.3	20.1	11	10.5	4.1	3.8
21	6.2	6.5	47	49	57	62	19	20	10	10	4	3.6
22	6.5	6.2	29	28	63	67	18	18.5	11.5	9	4.2	3
23	5.6	5.3	35	32	57	58	21	20.3	7	6.5	2.2	2
24	5	6	42	43	56	62	19	20.5	11	9.5	2.3	2
25	8	8.7	74	72	61	64	21	22	9.5	8	1.4	1.5
26	5.2	6.2	78	74	56	57	18.2	18.5	10.5	9	3	3.2
27	7	6.2	28.5	27	64	60	18	18.3	7	8.5	2.1	2.2
28	7	7.1	37	38.5	70	70	22.6	22	9	8.5	2	2
29	9	9	27	31	67	70	22	22	6	5	1.7	1.7
30	5	4.9	55	54	55	57	18.5	19	10	9	3.3	3

A: Prior to HU therapy B: value after 1 yrs completion of placebo threrapy

Table-6: Clinical effect of HU. N=32

A. Blood Transfusion:	No of Pts. in Grp-I (S)
i) Not needed	O5
ii). Less amount needed with prolonging interval	08
iii) Needed as before	19
B. Toxicity	
i). Severe anaemia with neutropenia	01
ii). Pain in the extremities	02
iii). Anorexia, headache & vertigo	01
iv). G. weakness & skin infection.	01

N.B: in control Grp B.T requirement as before & no toxicity

OBSERVATION

- All of the responded 13 patients were transfusion dependent prior to HU therapy.
- 5 (15.6%) pts needed no transfusion and 8 (25%) pts needed less amount of blood at prolonged intervals during study period than pre-HU therapy and which is statistically significant ($P<0.001$).
- No significant side effect of HU developed except one (bicytopenia developed).
- severe bicytopenia developed in one patient of the study group and excluded from the study group.
- Their growth, general well being and physical activity were found improved.

- Significant increased in Hb concentration of 7.0 ± 0.9 to 8.8 ± 1.0 gm/dl (mean 1.8 ± 0.3) in 13 patients.
- The Hb-F% increased in same 13 pts from 43.4 ± 14.8 to 59.3 ± 15.5% (mean 15.9 ± 3.4%)
- Reticulocyte count and total bilirubin concentration showed a significant decreased during HU therapy suggesting a decreased in haemolysis as a result of HU therapy.
- In E/B thalassaemia response were in 13 pts out of 25 (48%) but in β-thalassaemia major the response was 1 in 7 pts (14.3%), which was low in compare to other studies, may be due to low dose of HU or any genetic elements, nutritional factor, poor adherence to the treatment regimen.

CONCLUSION

- Our data support the efficacy of HU in decreasing the severity of anaemia and thus reducing or eliminating the requirement of blood transfusion.
- HU is more effective in Hb E/β thalassaemia than β-thalassaemia major.
- DNA analysis to detect the nature of genetic mutation causing thalassaemia & haemoglobinopathies to be done, so that it can be postulated that what type of mutation/mutations are better responsive to HU.
- Short & middle term tolerance are good and toxicities are minimum, though long term effects in children are still unknown.

Thank You