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Original Research :

Extended family screening of thalassemic children- To evaluate cost effective tests DCIP and NESTROFT

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Keywards :

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Abstract :

In this prospective observational study, 309 subjects who were the family members of thalassemia patients have been tested. All the subjects were divided into 4 groups. Group i)117 subjects had both tests positive, Group ii) 66 subjects had DCIP test negative and NESTROFT TEST positive Group iii) 12 subjects had DCIP test positive and NESTROFT test negative and Group iv)114 subjects had both tests negative. HPLC was done in all subjects for confirmation as a gold standard.

Out of 309 subjects 195 showed positive results with either or both the tests who also revealed abnormalities in HPLC. 114 subjects showed negative results with both DCIP and NESTROFT who were also normal in HPLC. The sensitivity of the combined tests is 100 percent and specificity is 92.68 percent.

Predictive value of positive test is 95.38 percent and Predictive value of negative test is 100 percent.

Introduction:

Hemoglobinopathy is one of the major problems in Indian childhood population. About 2-17% of Indian population with an average of 3% in the general population are thalassemia carriers[1]. The people during the carrier state usually looks normal and lead almost normal life. The disease is manifested when two carriers get married and give birth to a child with thalassemia major. There is still no definite curative treatment for thalassemia.Stem cell transfusion has been done with controversial results in regard to long term prognosis.Bone marrow transplantation remains within the reach of only a few affluent people. Those who are born with major hemoglobinopathy are treated with regular blood transfusion and chelation therapy. Regular blood transfusion is hazardous and painful affair for the child. When the child develops iron overload he is to be treated with chelation therapy. The treatment regime is very costly and beyond the reaches of the common people .The way to prevent the occurrence of the disease is the identification of the carrier and pre marital counselling.

The available tests for diagnosis and Screening require cell counter and HPLC.Both these methods are very costly. These facilities are available in apex hospitals and district hospitals. This is beyond the reach of the common people. Therefore mass screening cannot be done in such a way. The primary screening should be done by very low cost, easily available methods. The sample has to be collected from the door steps. Those who are primary screening test positive, their samples have to be re tested by cell counter and HPLC.

Screening should be done at the level of colleges, schools and different rural areas at the panchayat level. The samples need to be collected from the doorsteps of the people without waiting

for them to reach apex centres. The blood samples are to be screened by simple process and finally go for final analysis by cell counter and High Performance Liquid Chromatography(HPLC).

Modified Dichlorophenolindophenol (DCIP) test and Naked Eye Single Tube Osmotic Fragility Test(NESTROFT) are simple screening procedures for thalassemias which can have great importance in Indian senario.

The purpose of this study is to evaluate a simple screening strategy to detect the carrier of thalassemia and other hemoglobinopathies carrier at community level.

Objective:

To evaluate a screening strategy for detection of Thalassemia in rural areas with limited laboratory services.

Inclusion criteria:

Children in family of thalassemic patients with age 6 months to 15 years

Exclusion criteria:

Children with any other hemoglobinopathy or immunodefficiency

Materials & Methods:

Blood samples were collected from 309 subjects who were attending the Thalassemia clinic at Burdwan Medical College for extended family screening. The samples were tested by using combined Dichlorophenolindophenol precipitation test (DCIP) and NESTROFT tests. The final confirmation of the results was done by cell counter and High Performance Liquid Chromatography (HPLC).

Study technique :

(1) DCIP Precipitation Test – 20 microlitre of fresh blood collected in EDTA vials was mixed with 2ml of DCIP solution. The mixture is incubated at 37°C for 15 minutes. Test is interpreted as negative or positive by visualisation. Negative sample are clear and positive samples are cloudy.

(2) NESTROFT Test – 2ml of 0.36% saline in a glass tube was mixed with 20 micro litre of fresh blood collected in EDTA. The contents were mixed by inverting the tube and allowed to stand at room temperature for 30 min. The results were interpreted as positive or negative by visualization of sharp black line on a paper held not more than 1 cm behind the tube.

All 309 cases were evaluated by cell counter and HPLC.

Results and analysis:

The results of the combined tests on the blood samples of 309 subjects have been evaluated by reports of cell counter (Hb%, MCV, MCH, RDW) and HLPC (area curve of HbA2, HbF, HbA). The evaluation has been compiled in tables.

Table No-1 : Distribution of study subjects according to test results. (n=309).

Number (%)	DCIP	NESTROFT
117 (37.86)	+	+
66 (21.36)	-	+
12 (3.88)	+	-
114 (36.89)	-	-

Out of 309 study subjects 117 (37.86%) were positive for both the tests. 66 (21.36%) were only NESTROFT test positive, 12(3.88%) were only DCIP test positive. Thus 114(36.89%) subjects were negative for the tests. Thus, either one or both of the tests were positive in 195 (63%) subjects.

Table No - 2 : Distribution of haemoglobin levels according to test results (DCIP/NESTROFT). (n=309).

Hb g/dl level	+/+	-/+	+/-	-/-
6-8	2	2	1	7
>8-10	75	22	5	37
>10	40	42	6	70
Total	117	66	12	114

The table shows that large number of the carriers have haemoglobin level <10 g/dl. Thus the thalassemic family has high percentage of anemia in comparisons to normal population.

Table 3:The table shows relation between MCV level and test results. (DCIP/NESTROFT). (n=309).

	+/+	-/+	+/-	-/-
MCV<80fl	96(82%)	59(89.39%)	8(66.66)	11(9.64%)
MVC>80fl	21(17.94%)	7(10%)	4(33.33%)	103(90.35%)
Total	117	66	12	114

The table shows that majority of thalassemia carriers have MCV levels less than80fl. MCV levels are less than 80fl in 82% cases who are positive for both the tests. MCV level less than 80fl in 89.39% subjects who are only NESTROFT test positive.MCV level less than 80fl in 66.66% subjects who where only DCIP test positive. Whereas 90.35% of normal population had MCV levels more than 80fl.

Table No –4: relation between MCH level and test results. (DCIP/NESTROFT). (n=309).

		+/+	-/+	+/--/-
MCH<27pg	100(85.54%)	61(92.42%)	10(83.33)	50(43.85%)
MCH>27pg	17(14.52%)	5((7.57%)	2(16.66%)	64(56.14%)
	117	66	12	114

The table shows that majority of thalassemia carriers have MCH levels less than 27pg. MCH levels are less than 27pg. in 85.54% subjects who were positive for both the tests.MCV level less than27pg.in 92.42% subjects who were only NESTROFT test positive.MCV level less than 27pg. in 83.33% subjects who where only DCIP test positive. Whereas 56% of normal population had MCH levels more than 27pg.

Table No -5: relation between RDW value and test results. (DCIP/NESTROFT). (n=309).

	+/+	-/+	+/-	-/-
RDW(11.5-14.5)	36(30.76%)	21(31.81%)	8((66.66)\ %)	48((42.21%)
RDW>14.5	81(69%)	45(68%)	4(33.33%)	66(57.89%)
	117	66	12	114

The table shows that the percentage of subjects with high RDW (>14.5) are more in those who are positive for the combined tests and positive for NESTROFT test. Thus RDW is high in case of thalassemia carriers. Low MCH and high RDW is very much suspicious of hemoglobinopathy.

Table No -6: Results of DCIP test and statistical analysis of data (n=309)After confirmation by HPLC

	No. of Subjects with Thalassemia carrier state	No. of Subjects without Thalassemia carrier state	Total
DCIP(+)	129	0	129
DCIP(-)	66	114	180
	195	114	309

Sensitivity is 66.15%
Specificity is 100%
Predictive value of positive test is 100%
Predictive value of negative test is 63.33%

Table No -7 : Results of NESTROFT test and statistical analysis of data (n=309) After confirmation by HPLC

	No. of Subjects with Thalassemia Carrier	No. of Subjects without Thalassemia Carrier	Total
NESTROFT(+)	183	0	183
NESTROFT(-)	12	114	126
	195	114	309

Sensitivity is 93.84%
Specificity is 100%
Predictive value of positive test is 100%
Predictive value of negative test is 90.47%

It is observed from table no.6 & table no.7 that the sensitivity and Predictive value of negative test are higher for NESTRFOT test than that of DCIP test.

Table No - 8 :Results of combined tests and statistical analysis of data (n=309) After confirmation of by HPLC.

Combined tests	No. of Subjects with Thalassemia carrier state	No. of Subjects without Thalassemia carrier state	Total
Both tests or either test positive	195	0	195
Both tests negative	0	114	114
	195	114	309

Sensitivity is 100%
Specificity is 100%
Predictive value of positive test is 100%
Predictive value of negative test is 100%

The study had 195 carriers out of 309 screened subjects. All the carriers had either both (NESTROFT and DCIP) tests positive or any one of the tests positive (NESTROFT/DCIP).

Discussion :

This study has been done to find out a simple and inexpensive methodology for primary screening of thalassemia carrier in urban and rural areas where the health facilities are limited and also been utilised in vast population survey. The families of thalassemia carrier and wide spectrum of their families are purposely included so that the positivity of the test will be more and the authenticity of the test will be better judged. NESTROFT is positive in thalssemia and thalassemia carriers whereas DCIP is positive in E thalassemia or any unstable hemoglibinopathy. Thus combinations of two tests will help to detect the thalssemia carrier and associated any unstable hemoglobinopathy or carriers.

Conventionally primary screening for all forms of thalassemia relies on haematological index cut-offs which involves an electronic cell counter. Individuals with MCV less than 80fl needs further investigation to exclude thalassemia disease or carrier. In the present study MCV level less than 80fl in cases of positive NESTROFT is 89.39% whereas in cases of positive DCIP is 66.66%. But in combination tests it is 82%.

There is high positivity of NESTROFT test because this test is positive in any hemoglobinopathy whereas DCIP is positive only in E hemoglobinopathy or any unstable hemoglobinopathy. Thus in present series sensitivity of DCIP test is less.

MCH below 27pg. should be examined further to confirm or exclude the diagnosis of thalassemia[3]. In the present study MCH level is less than 27pg. in 92.42% of NESTROFT positive subjects, 83.33% of DCIP positive subjects. But in combination tests it is 85.54%, the combined percentage is less because the positivity of DCIP is less because of the same reasons described before.

In case of hemoglobinopathy RDW is usually more than normal. In the present study, RDW is high in 33.33% of DCIP positive cases, 68% of NOSTROFT positive cases, and 69 % of cases with both tests positive. If any individual has low MCV and high RDW, he always needs thalassemia screening.

In the present study there is high percentage of low MCV and increase value of RDW in case of NESTROFT and combined tests positive subjects but not in case of DCIP positive subjects. It is due higher haemoglobin level and less number of cases in this group.

A simple visual test for the detection of haemoglobin E has been proposed, based on a observation when a blue dye, 2,6 dichlorophenolindophenol (DCIP) was used in testing for red cell enzyme deficiencies[4,5]. It was noted that solutions containing haemoglobin E (from patients with either haemoglobin E heterozygosity or homozygosity) became turbid when exposed to this dye. This test has been used in Thailand for antenatal screening in under resourced areas [6, 7,] but there are other countries with a high prevalence of haemoglobin E where routine antenatal screening for this variant haemoglobin is not carried out. The test as initially described has been modified and is now available in kit form[8].

Microcytic hypochromic red cells of thalassemia trait are more resistant to lysis in 0.36% saline solution than normal normochromic normocytic red cells. This test is called NESTROFT Test. This test is being widely used in India and other developing countries in thalassemia screening programmes[9]

In a study conducted on 301Thai-Khmer participants, who were screened for thalassemia and HbE using combined osmotic fragility and modified DCIP tests. Sensitivity, specificity, positive and negative predictive values for the combined tests were 100%, 69.8%, 77.2% and 100%[10].

Our study however had higher values for

specificity and positive predictive values. The rates for sensitivity and specificity in present study are higher because the samples are from the extended family members. It is very good observation that the family with thalssemia is to be screened before marriage.

Another study was conducted on 587 HbE and 280 reference subjects in a study conducted in Thailand. In this study combined osmotic fragility and modified DCIP tests had sensitivity, specificity, positive and negative predictive values as 99.43%79.29%, 90.03% and 96.67%[11].

In the present study DCIP sensitivity is 66.15% specificity is 100%.

Predictive value of positive test is 100% predictive value of negative test is 63.33% whereas in case of NESTROT sensitivity is 93.84% specificity is 100%, predictive value of positive test is 100% Predictive value of negative test is 90.47%.

In case of combined tests sensitivity is 100%, specificity is 100%, Predictive value of positive test is 100%, predictive value of negative test is 100%. In the present study there is higher percentage of positive value because the subjects are taken from extended thalassemia families.It is still advisable to these screening tests ,even in family members to reduce the cost involved in HPLC.

Conclusion :

NESTROFT and DCIP are good preliminary tests for screening of thalassemia carriers. The final confirmation should be done by HPLC.This tes is very and cost effective approach which can be done in rural areas and mass screening before going for highly costly HPLC method.

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