Assessment of Adrenal Endocrine Function in Asian Thalassemics

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Adrenal endocrine function was assessed in a cohort of 20 patients, between 10 and 20 years of age, with transfusion dependent beta thalassemia. Cortisol levels were assayed before and after ACTH stimulation with 1µg and 250 µg. Adrenal dysfunction was defined as a basal cortisol of <400 nmol/L and/or peak cortisol levels of <500 nmol/L. Overall, 9 patients (45%) had in vitro evidence of adrenal dysfunction. A statistical significant correlation (r=0.4308; P<0.05), between wasting and the basal cortisol level, was observed. Similarly, there was correlation between the number of transfusions received and growth failure (r=0.4774; P<0.05). In comparison to the involvement of other endocrine axes in polytransfused thalassemics, the adrenal endocrine function abnormalities are minor and clinically of little consequence. The observations, albeit, in a small cohort of thalassemics, stress the need for an annual estimation of basal cortisol level, especially in patients with wasting.

Keywords: Adrenal, endocrine, iron overload, thalassemia.

Asian thalassemics are unique in the sense that financial, political and social circumstances continue to deny prompt diagnosis and ideal management to them. The majority end up receiving regular packed cell transfusions, alone, to maintain satisfactory hemoglobin levels. A substantial proportion cannot afford iron chelation. The consequent overload of iron that occurs damages various organs(1). Significant endocrine dysfunction is well documented with particular affection of the pituitary, pancreas, thyroid and parathyroid glands(2). In contrast, there are only a few studies on adrenal function in b-thalassemia, especially in Asia(3-8). The methodology, in the previous studies did not include the recently approved 1µg ACTH and 250µg ACTH stimulation tests. Further, the observations and results have been inconsistent(3-8). The implications of hypofunction of the adrenals in a child with an already compromised homeostasis due to chronic anemia can be serious. This study was designed to assess adrenal dysfunction in Asian thalassemics.

Subjects and Methods

Patients with transfusion dependent beta thalassemia who fulfilled the inclusion criteria were recruited for the study.

Inclusion criteria: (a) Diagnosis already established by examination of the peripheral blood film, estimation of HbF, hemoglobin electrophoresis and parental studies at initial registration; (b) onset of transfusion therapy before the age of 2 years; (c) transfusion requirement at least once in 4-6 weeks; (d) age more than 10 years; and (e) at least 21 days elapsed from last transfusion.

Exclusion criteria: (a) History of recent infection or any other stress within the
preceding 4 weeks; (b) overt evidence of congestive cardiac failure, hepatic dysfunction, diabetes; and (c) intake of drugs affecting adrenal function (antitubercular drugs, anticonvulsants, ketoconazole, etc.).

One hundred ten of the 350 ß-thalassemics enrolled in the Thalassemia transfusion program satisfied the inclusion criteria. A computer generated random list of 20 out of these 110 cases. Informed consent was obtained from these subjects and their parents before enrolment. The Institute’s Ethics Committee approved the study.

On day one, at 8.00 a.m., a blood sample for cortisol was drawn (0 min), following which 1 µg dose of ACTH was injected IV and samples for cortisol were drawn at 30 min and 60 min after the injection. On day 2, the same procedure was repeated with 250 µg of ACTH and samples for cortisol were drawn before (0 min), 30 min and 60 min after the injection. Serum cortisol levels were assayed using standard radioimmunoassay(9). Blood for serum ferritin levels were drawn on day 1 of the test protocol and ferritin was assayed using enzyme immunoassay(10).

A baseline value (0 min), of less than 400 nmol/L, was considered abnormal. The reason for choosing this rather high value was based on the following facts (a) the study subjects are stressed to a certain extent due to the presence of chronic anemia with consequent hypoxia; and (b) the hospital admission and the insertion of the IV line. A cortisol value of 500 nmol/L completely separates patients with known adrenal insufficiency from normal controls(11). The stimulated cortisol responses (30 min, 60 min) of patients during low dose and standard dose ACTH stimulation test protocols were compared with standard reference guidelines(12,13). Even though the 250 µg ACTH test is still considered the gold standard for assessing adrenal reserve and diagnosing primary adrenal insufficiency. In the last few years, several workers have documented that the circulating levels of ACTH achieved by injecting a 1µg dose are equivalent to that achieved by insulin hypoglycemia or surgical stress(14). Hence, the 1 µg test has largely replaced the insulin hypoglycemia test for assessing the hypothalmic-pituitary-adrenal (HPA) axis and to diagnose secondary adrenal insufficiency.

The chi-square test was used for categorical data, the Mann-Whitney U-test for ordinal data and the Pearson’s correlation coefficient for continuous variables. P <0.05 was considered significant.

**Results**

The salient features of the patients with or without adrenal dysfunction are shown in Table I. The basal and the ACTH stimulated cortisol responses are also depicted in Table I. The stimulated response was low only in stray cases. One subject failed the 1µg ACTH test and another patient failed the 250µg ACTH test.

The height percentage for age and weight percentage for age of the subjects were compared with total transfusions received. The correlation coefficient values were –0.4308 and –0.4774, respectively, which were statistically significant (P <0.05). The weight percentage for age of the subjects was correlated with absolute cortisol values (Fig. 1). The correlation coefficient value was 0.4308, which was statistically significant (p <0.05). Serum ferritin values ranged from 480 to 8800 µg/L with a mean of 3834 ± 2134 µg/L. When the chelation status was compared with the incidence of low baseline cortisol, the value of chi-square was 0.0824, which was not significant (P >0.1).
TABLE 1–Salient Features and Cortisol Levels (mean ± SD) in Thalassemics with or Without Adrenal Dysfunction.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Feature</th>
<th>Dysfunction (n = 9)</th>
<th>Normal function (n = 11)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Age (yr)</td>
<td>14.6 ± 3.4</td>
<td>12.9 ± 2.3</td>
<td>0.309</td>
</tr>
<tr>
<td>2.</td>
<td>Duration of transfusion (mo)</td>
<td>164 ± 40</td>
<td>149 ± 26</td>
<td>0.310</td>
</tr>
<tr>
<td>3.</td>
<td>Total no. of transfusions</td>
<td>248 ± 112</td>
<td>226 ± 50</td>
<td>0.539</td>
</tr>
<tr>
<td>4.</td>
<td>Units of transfusion(n)</td>
<td>1.49 ± 0.5</td>
<td>1.51 ± 1.2</td>
<td>0.914</td>
</tr>
<tr>
<td>5.</td>
<td>S. Ferritin (ng/mL)</td>
<td>4342 ± 2619</td>
<td>3418 ± 1655</td>
<td>1.011</td>
</tr>
<tr>
<td>6.</td>
<td>Cortisol level (nmol/L) baseline</td>
<td>308 ± 89</td>
<td>626 ± 178</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>1µg ACTH (30 min)</td>
<td>587 ± 269</td>
<td>962 ± 208</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>1µg ACTH (60 min)</td>
<td>648 ± 306</td>
<td>913 ± 193</td>
<td>0.044</td>
</tr>
<tr>
<td></td>
<td>250 µg ACTH (30 min)</td>
<td>589 ± 220</td>
<td>1059 ± 122</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>250 µg ACTH (60 min)</td>
<td>618 ± 143</td>
<td>1025 ± 173</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Discussion

In our study, nine (45%) of 20 patients with thalassemia were found to have abnormally low baseline cortisol. Most studies on adrenal function in thalassemics were carried out before the newer tests for assessing adrenal function were developed (4-8). Earlier investigators had used a variety of methods to assess adrenal function e.g., 17-ketosteroid estimation in urine, 17-ketogenic steroid assays and plasma renin activity. All these assays are of limited utility in evaluating adrenal function.

McIntosh, et al. (2) studied 6 patients with

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*Fig. 1. Correlation between baseline serum cortisol and failure to thrive.*
thalassemia major. Cortisol response to insulin-induced hypoglycaemia was normal in all. There was also normal cortisol response to ACTH. However, they documented raised ACTH values in all the cases and suggested that this could be due to end organ unresponsiveness. Costin, et al. (4) studied 16 patients with thalassemia. They found subnormal cortisol responses to insulin-induced hypoglycemia in 3 (18.7%) patients. ACTH values were abnormal in 5 (32%) and the cortisol response to ACTH was also subnormal in 5 (32%). Hazmi, et al. (7) studied 44 patients with thalassemia and found lower mean plasma cortisol levels in the patients in comparison to controls and the difference was statistically significant. In addition, they observed a negative correlation between plasma ferritin and mean cortisol levels in all their patients. Sklar, et al. (3) evaluated 8 patients, all of whom had a normal cortisol response to prolonged ACTH infusion. However, the peak dihydroepiandrostenodione (DHEA) levels were lower than the values observed in controls; the difference was statistically significant (3). Bashir, et al. (8) investigated 22 patients with thalassemia and found that the mean cortisol levels were significantly lower in all cases when compared to controls.

There was a significant correlation between the degree of wasting and stunting and total transfusions received, indicating that the more the number of transfusions received, the lesser the height and weight for age. The red cell transfusions increase the iron overload which, in turn, affects the endocrine function of the pituitary causing decreased height and weight. The excess iron has the potential to disrupt adrenal function which results in low cortisol levels. Serum ferritin values correlated, poorly, with cortisol values. This mirrors the observations of several other workers who found that serum ferritin is not a reliable indicator of iron overload in thalassemics, especially in poorly chelated patients, since it is an acute phase reactant and can also be elevated by hepatic injury (16). In our study, one patient failed the 1µg ACTH test, while responding to the standard 250 µg test, indicating a subtle abnormality of the hypothalamic-pituitary adrenal axis at hypothalamic or pituitary or adrenal level where a normal response is observed with a supraphysiological stimulation and a blunted response manifests with a physiological low dose (1µg) of ACTH (14-16).

To conclude, we have documented subtle abnormalities of adrenocortical function in the form of low baseline cortisol, though the stimulated cortisol response is largely preserved. Considering the magnitude of other endocrine axes involved in thalassemia due to iron overload, namely the hypothalamic-pituitary-gonadal axis and the GHRH-GH-IGF-1 axis, the adrenal functional abnormalities are minor and clinically insignificant. This might have a teleological
explanation, where homeostasis is designed to preserve the life saving hormone cortisol, even in the most adverse circumstances.

Contributors: AS was primarily responsible for patient screening, enrolment, data collection, preliminary analysis and writing the manuscript. RM and AT participated in the development of the protocol and analytic framework of the study, and contributed to the writing of the manuscript. RKM supervised the design and execution of the study, performed the final data analysis and was involved in overseeing the final manuscript.

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REFERENCES