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Anterior pituitary gland assessment in sickle cell anaemia patients with delayed menarche

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Summary
Pituitary gland dysfunction and its contribution to menarcheal delay in sickle cell anaemia patients was investigated. Ten SS patients mean age 17.5 years who had not achieved menarche were recruited and 10 each of AS and AA controls, mean ages 17.4 and 17.7 years were used as controls to study the effect of the heterozygous state. Dynamic studies with LHRH and TRH were performed for 60 minutes and LH, FSH, PRL and TSH assays were done. Median basal values were significantly lower in the SS patients compared with the AS and AA controls for LH, FSH and PRL. LH: 3.0; 7.1; 7.7 U/L, FSH: 2.1; 4.3; 5.1 U/L. PRL: 94.5; 590; 390 U/L, respectively. The median basal TSH values did not show any significant difference between the SS subjects (7.3 U/L) and the AS and AA controls (5.4 U/L) and 5.6 U/L, respectively. The readily releasable pool also showed the same pattern for LH, FSH and PRL as the basal values while the SS subjects had higher median TSH releasable pool values that were significantly different from those of the AA controls. From the prolactin responses three subjects demonstrated maturational delay in menarcheal achievement while seven demonstrated isolated gonadotrophin deficiency. It is concluded that SS patients with delayed menarche have a hypothalamo-pituitary axis dysfunction that gives rise to delay in menarcheal achievement and metabolic adaptations to stress of illness. The heterozygous state did not delay menarcheal onset.

Keywords
Pituitary Gland, Delayed Menarche, Sickle Cell Anaemia

Résumé
Le disfonctionement de la glande pituitaire et la contribution au retard du menarch chez les patients drepanociaires a été invstiguer. Dix patients 35 d'age moyen 17,5 ans qui n'avaient pas encore atteint le menarche avaient ete recruite. Dix patients dans chacun des groupes AS et AA, age moyen 17,4 ans et 17,7 ans avaient ete utilisés comme controle afin d'etudier l'etat heterozygote. Des etudes dynamiques avec LHRH, et la TRH avaient ete faite pendant 60 minutes et les assays de la LH, la FSH, la PRL et la TSH avaient ete faite egalement. Les valeurs mediianes de bases avaient ete significativement faible chez les patients SS compare aux controless AS et AA pour les assays de la LH, FSH et PRL. LH: 3,0; 7,1; 7,7 u/l, FSH: 2,1; 4,3; 5, 1 u/l PRL: 94, 5; 590; 390 u/l respectivement. La moyenne des valuers de base de la TSH n' avait montré aucune valuer differents significative entre les sujects SS avait en une forte valeur moyenne de pool liberable de la TSH qui etait significativement different des controles.

Des reponses de la prolactine, 3 sujets avaient demontres une deficience isole en Gonadotrophine. II avait ete conclut que les patients SS ayant un retard du menarche ont une malfonction de l'axe hypothalemo-pituitaire, qui resulte a un retard dans l'atteinte du menarche et des adaptations metaboliques au stress des maladies. Les heterozygotes n'avaient pax retarde le declanchement memarcheal.

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Introduction

The sickle cell gene is widely distributed in tropical Africa, the Mediterranean, the Arabian Peninsula and parts of India. In Nigeria, about twenty-five per cent of the population has the sickle cell trait (1,2) and about two per cent suffer from sickle cell anaemia [1,3,4]. The clinical phenotype of sickle cell anaemia is severe. Children and adolescents with this disorder have a reduction in anthropometric indices and delayed menarche [5,6,7]. Delay in menarche has been attributed to constitutional delay in adolescence [8,9] and primary and secondary hypognonadism [10,11]. Cerebral infarcts, haemorrhage and emboli are complications of the sickling phenomenon especially in children, which impact on the hypothalamus and the pituitary gland [12]. We have therefore set out in this study to assess the anterior pituitary gland in this setting and how this and the presence of the S gene contribute to delayed menarche.

Methods

Subjects and controls

Ten sickle cell anaemia patients within the age bracket of 16-18 years (the age bracket when any girl who has not achieved menarche is evaluated) and who had not achieved menarche were recruited from the Haematology Clinic of the University College Hospital (UCH), Ibadan, Nigeria. They were all Tanner Stage P2 and had been clinically stable three months before the test procedure.

Premenarcheal AA and AS Tanner State 2 girls could not be used as controls because of socio-cultural factors in this environment. We therefore used age-matched AA and AS controls from the same socio-cultural background. Informed consent was obtained from patients, controls and their parents.

Test procedure

The tests were performed in the morning at the Metabolic Research Unit of the UCH. Genotypes were ascertained by cellulose acetate haemoglobin electrophoresis. A general physical examination was done to ascertain well-being and Tanner staging. Controls were studied within five days of cessation of menstruation. Heights and weights were also recorded

Blood samples were obtained in the supine position from a large antecubital vein 15 minutes after cannula insertion for basal hormone measurements.

Intravenous bolus injections of 100 ug luteinizing hormone releasing hormone (LHRH) and 200 ug of thyrotropin releasing hormone (TRH) (both obtained from Hoechst AG, Frankfurt, Germany) were then given and subsequent samples were collected 30 minutes and 60 minutes later. Samples were collected into heparinized tubes and centrifuged immediately. The plasma was stored frozen at −20°C before estimation within one month of collection.

Analytical methods

Plasma levels of luteininzing hormone (LH), follicle stimulating hormone (FSH) and prolactin were determined by standard radioimmunoassay (RIA) techniques (World Health Organisation Matched Assay Reagents for the Special Programme of Research in Human Reproduction). The standards for the gonadotrophin RIA were calibrated against the WHO Second International Preparation (IRP) of pituitary FSH/LH 78/549; prolactin standards were calibrated against WHO IRP Prolactin 75/504. Methodological details are as set out in the manual. Our laboratory is a centre for collaborative research with the WHO programme. Female reference values for LH range from 2.4 to 10 U/L; FSH from 2.0 to 7.0 U/L and for prolactin from 120 to 750 U/L. Intra-assay coefficients of variation for LH, FSH and prolactin were 4.9%, 4.1% and 2.5% at 5 U/L, 5 U/L and 500 U/L, respectively. The minimal detection concentrations for FSH, LH and PRL were 0.8 U/L, 1.0 U/L, and 50 U/L, respectively. Thyrotrophin stimulating hormone (TSH) was determined by immunoradiometric assay (Diagnostic Products Corporation, Los Angeles, CA, USA). The standards were calibrated against WHO 2nd IRP 80/558. Reference values for TSH range from 0.3 to 5 U/L with a minimal detection concentration of 0.1 U/L. Intrassay coefficient of variation was 3.4% at 2 U/L.

Statistical analyses

Incremental areas under the 60 min. hormone concentration time curve were calculated by the trapezoidal rule for each hormone. The differences between (1) the basal values and (2) the incremental areas for each hormone between subjects and controls were sought by the nonparameric method of Mann-Whitney U because of the small sample size and to allow for nonnormality of distribution. The MINITAB software program was used. The level of significance was $P<0.05$.

Results

The results are summarized in Tables 1,2 & Fig. 1 a,b,c,d.

Basal value

The AA and AS controls had significantly higher median basal values that the SS subjects for LH, FSH, and PRL. It is noteworthy that the SS subjects' median basal values for LH (3.0 U/L) and FSH (2.1 u/L) are low normal and subnormal for PRL (94.5 U/L) with our laboratory reference values. The TSH values were not significantly higher in the AA and AS controls when compared with the SS subjects. There was no difference in all the hormones between AS and AA controls.

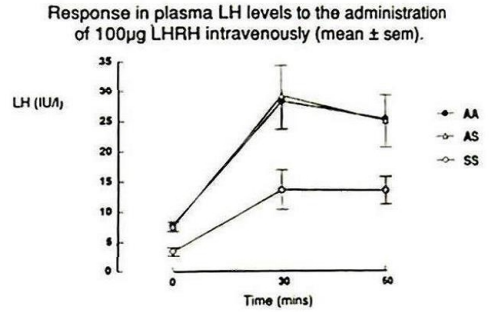

Fig. 1a

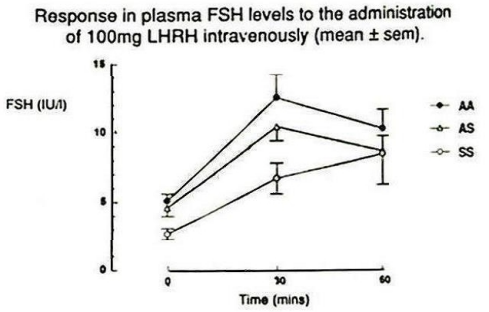

Fig. 1b.

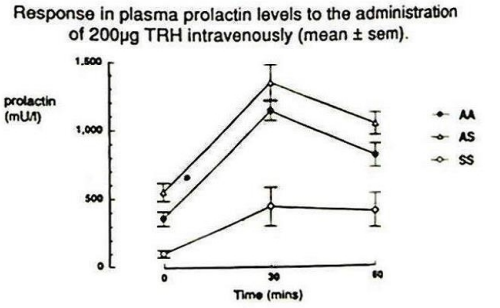

Fig. 1c

Table 1: Basal hormone values median (Interquartile Range) U/L

	LH	FSH	PRL	TSH
SS	3.0 (1.7 – 4.7)	2.1 (1.7 – 3.6)	94.5 (48.5 – 148.0)	7.3 (5.1 - 7.7)
AS	7.1 (5.1 – 9.6)^{x}	4.3(3.7 – 6.0)*	590.0 (383.7 – 746.2)$^{@}$	5.4 (4.0 – 6.0)
AA	7.7 (6.6 – 9.0)$^{+}$	5.1 (3.9 – 6.2)$^{-}$	390.0 (241.0 – 0-505.0)†	5.6(4.6 - 6.1)

XP = 0.0014 AS vs SS; $^{*}P$ = 0.0111 AS vs SS; $@P$ = 0.006 AS vs SS
$+P$ = 0.00116 AA vs SS; $-P$ = 0.0014 AA vs SS; $\dagger P$ = 0.0036 AA vs SS

Table 2: Incremental Hormone Values Median (Interquartile Range) U/L. Min.

	LH	FSH	PRL	TSH
SS	549.0 (285.0 – 1078.5)	314.5 (256.5 – 379.5)	11835 (7650 – 41460)	1898.5 (1588.5 – 2116.5)
AS	1129.0 (1055.0 – 1728.0)*	474.5 (450.0 – 634.5)*	64387 (57150 – 71925)^{T}	1095.0 (978.0 – 2007.0)
AA	1061.0 (928.5 – 1755.0)$^{+}$	547.0 (425.0 – 740.0)$^{@}$	53100 (4475 – 56325)^{1,0}	1333.5 (1110.0 – 1617.0)$^{-}$

$^{*}P$ = 0.017 AS vs SS; $^{\bullet}P$ = 0.009 AS vs SS; ^{T}P = 0.00003 AS vs SS; ^{-}P = 0.04 AA vs SS
^{+}P = 0.021 AA vs SS; $^{@}P$ = 0.041 AA vs SS; ^{3}P = 0.0003 AA vs SS
^{O}P = 0.04 AA vs AS

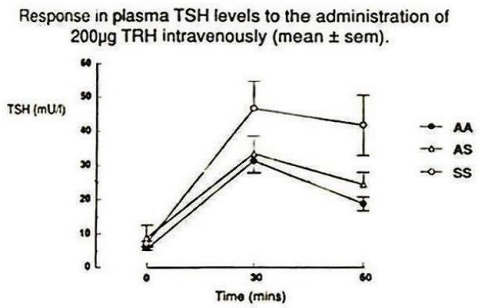

Fig. 1d

Incremental Value (Table 2)

These values, obtained by the LHRH and TRH stimulation tests, are useful for assessing anterior pituitary hormone reserve (readily releasable pool) for LH, FSH, PRL, and TSH. The AA and AS controls also show significantly higher median values than the SS subjects for LH, FSH and PRL. On the other hand, the TSH response showed an opposing pattern. The SS subjects had higher median TSH incremental values than AA and AS subjects, but this was only significant when compared with AA controls. There was no difference between AA and AS controls for LH, FSH and TSH except for PRL where AS median values were significantly higher (P = 0.04).

From Fig. 1, a, b,c,d, mean peak reponses of LH, PRL, TSH were observed for all the study groups at 30 mins and in each case there was a significant lower difference between the subjects (SS) and the controls (AA & AS). However there are two variations. The FSH response to LHRH stimulation continued to rise at 60 mins for the SS subjects and the mean peak response of TSH to TRH stimulation was significantly higher in the SS subjects than AA and AS controls.

A plot of the individual (patients and control) prolactin responses to TRH stimulation (graphs not shown) demonstrated that three SS subjects had similar responses as AA and AS controls while the remaining SS subjects demonstrated flat curves.

The SS subjects weighed significantly less than the AA and AS controls [mean (S.E.) in kg]: SS = 31.6 (1.6); AA = 51.6 (2.2); AS = 53.9 (3.1) PL = (0.5). The mean (SE) ages in years at menarche for the controls were similar: AA = 13.0 (0.3); AS = 12.3 (0.5). The mean (SE) ages in years for subjects (SS) and controls (AA and AS) were also similar: SS = 17.5 (0.2); AA = 17.7 (0.1); AS = 17.4 (0.2).

Discussion

The dynamic stimulation studies in this cohort of subjects had demonstrated that there is a remarkable reduction in the anterior pituitary gland functional reserve capacity of sickle cell anaemia patients who have not achieved menarche well beyond the average age of menarche for unaffected peers. Mean age at menarche in SS patients is about 15.5 years and 13 years in the unaffected population [14].

Our controls have started menstruating and therefore have primed ovaries, but the immediate post-menstrual period is endocrinologically similar to Tanner Stage P2 subjects. Most girls at age 16 years who have not menstruated have pathological causes. Dickerman *et al* [17] in Israel had established norms for LH and FSH

responses to LHRH for the different stages of pubertal development – Tanner Stage 1 through 5 in boys and girls with AA genotype. In this study, the LH response to LHRH stimulation over 90 mins showed peak responses at 30 mins for P1–P5 with P1 having the smallest and P5 the highest. For FSH, peak response was at 60 mins and remained so to 90 mins. P1 had the least responses and P2 the highest (Fig. 2 a, b Dickerman's Graph).

Plasma LH (Fig 2a) and FSH (Fig 2b) responses to a one-bolus LH-RH injection (50 μg/sq m intravenously) in normal girls before puberty (P_1) and at various stages of puberty (P_{2-5}) mean ± SD, NS signifies nonsignificant; Fig 2a asterisk signifies $P \leq 0.1$ to 0.02) Adapted from Dickerman et al (17)

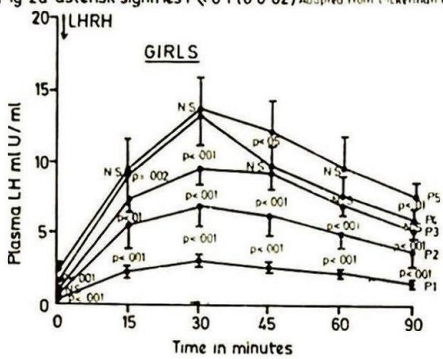

Fig. 2a

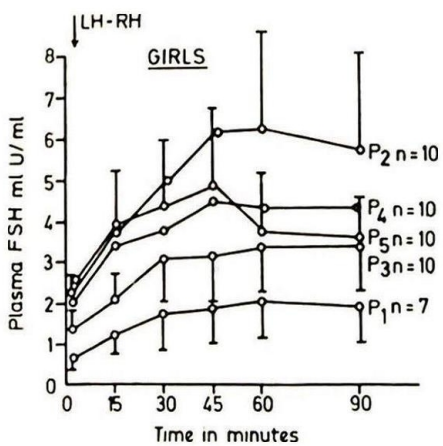

Fig. 2b

Thus, P2 responses most likely represent the poor priming stage of the hypothalamo-pituitary axis of our study patients.

The LH and FSH responses in our patients (both basal and incremental) are similar to those in Dikerman's study, the difference being that the subjects are older and have sickle cell anaemia. The response is in keeping with a dysfunction of the hypothalamic-pituitary axis which plays a role in delaying menarche.

Furthermore, Olambiwonnu *et al.* [8] in a cross sectional study of sickle cell anaemia patients with delayed puberty, and Luban *et al.* [9] in a longitudinal study in a similar group of patients showed appropriate gonadotrophin values for the stages of pubertal development which they concluded would be due to maturational variation in the HPG axis which in the usual finding in constitutional delay. Rothman *et al.* [12] have also demonstrated cerebral infarcts, haemorrhage and emboli by MRI as complications of the sickling phenomenon in children. By stimulating the pituitary gland for releasable hormone reserve, we have functionally demonstrated that the pituitary gland or the hypothalalmus may be the seat of these cerebral events. These events will eventually lead to poor "priming" of the pituitary gland to initiate and propagate normal pubertal developments in this cohort of patients. Adequate priming of the pituitary gland for menarcheal achievement depends on a delicate balance between CNS neurohomones, neurotransmitters, pituitary gonadotrophins and end organ oestradiol responses. Spitz *et al.* [15,16] have demonstrated that a prevailing low oestrogen state will fail to "prime" the pituitary gland and therefore be unable to maintain prolactin responsiveness in the achievement of menarche. This is in agreement with our findings where seven of our subjects demonstrated a flat prolactin response. This suggests isolated gonadotrophin deficiency. The three subjects that respond well to prolactin suggest a constitutional delay in maturation of the HP axis as also observed by Spitz *et al.* [16]. However, we did not estimate oestradiol values in these patients to confirm this.

We note that while basal TSH values did not differ significantly between the SS patients and the controls (AA and AS), the median incremental TSH responses for the SS subjects were significantly higher than those of AA controls. This TSH reponse to TRH stimulation in the SS patients is suggestive of a subclinical hypothalamic abnormality. This is supported by Rothman *et al.* [12] in their neuropathologic study.

The pattern that emerges is a gonadotrophic response among sickle cell anaemia patients with evidence of variable HP axis dysfunction and delayed menarche, which is similar to premenarcheal AA controls. However, there is a very significant time lapse (8 years vs. 17 years) which probably requires the S gene in the homozygous state to manifest.

In conclusion, the sickling phenomenon on the anterior pituitary gland and the HP axis in SS anaemia patients contributes to delayed menarche by giving rise to maturational delay of the HP axis or isolated gonadotrophin deficiency. The SS patients weighed significantly less (31.6 kg) when compared with the AA and AS controls (51.6 and 53.9 kg, respectively). Taken together with their characteristic lean body habitus, they expectedly will have a delay in achieving the "critical weight" of 47 kg and the appropriate lean mass to fat ratio that is required to achieve menarche. The contribution of chronic anaemia, energy cost of increased cardiovascular work and nutritional deficiencies to these factors will expectedly impact in delayed menarche in SS anaemia patients and the effects usually occur early in life.

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