Nigerian Journal of Physiological Sciences 23 (1-2): 61-66 ©Physiological Society of Nigeria, 2008
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THYROID HORMONE: A "PRIME SUSPECT" IN HUMAN IMMUNO DEFICIENCY VIRUS (HIV/AIDS) PATIENTS?

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Summary: Acquired Immunodeficiency Syndrome (AIDS) is the final and most serious stage of the disease caused by human immunodeficiency virus. The Immune system is the target of AIDS. We investigate presently any possible involvement of thyroid hormone, the deficiency of which gives rise to oedema and susceptibility to nonspecific infections; with a view to finding the primary factor seeding the disease. It has been reported that circumcision reduced the incidence of HIV/AIDS infection. Beyond circumcision however there might be some constitutional factor that comprises HIV infection to clinical AIDS. It is against this background that our research team turned to possible dyshormonopoisis and to thyroid hormone as a prime suspect among other possible factors that cause clinical AIDS. Moreover the hormone has been reported to be crucial for optimum immune function. A population of 200 seropositive AIDS patients were investigated against a control of 50 subjects made up of 25 healthy circumcised males and 25 healthy females; all of who were seronegative for the disease. The parameters investigated include thyrotropin (TSH), Thyroxine (T_4), Total protein (TP), Albumin (Alb), Globulin (Glob), Immune complex (IC_3) and Bence Jones proteins (BJP) levels in serum or urine. All seropositive clinically HIV/AIDS patients were hypothyroid. Seronegatives had significantly higher T4, TP, and Alb levels at $P<0.001$ and $P<0.05$ for Glob than seropositives. Seropositive females exhibited significantly ($P<0.001$) higher levels of IC3 than seronegative males. The globulin levels of all HIV patients were significantly ($P<0.05$) higher than control. BJP was also isolated in the urine of patients. The findings suggest that thyroid hormone deficiency is a primary culprit for the other inert or dormant factors to be activated.

Key Words: Thyroid Hormone, HIV/AIDS, TSH, T_4, Seropositive / Negative, Hypothyroidism.

Introduction

Human immunodeficiency Virus (HIV) infection is pandemic World Wide. The scourge is on the increase despite attempts by orthodox and herbal medical practitioners and scientists to stem it. Researches on seropositive patients have shown that thrombocytopaenia, and severe anaemia manifest as abnormally high erythrocyte sedimentation rate (ESR) (Sullivan 2002). It has been reported that circumcision reduced the incidence of HIV/AIDS infection (World Congress on HIV/AIDS 2007). Beyond circumcision however there might be some constitutional factor that converts HIV infection to clinical AIDS. It is against this background that our research team has turned to possible dyshormonopoiesis and to thyroid hormone as a prime suspect among other possible factors that cause clinical AIDS.

In an attempt to further elucidate more features of HIV disease which erupts as Acquired Immunoficiency Sydrome (AIDS) the present study was undertaken to investigate any possible involvement of thyroid hormone. The finding would probably identify its role or status in AIDS with a view to attending to the outcome. Thyroid hormone was speculated since hypothyroidism caused oedema of the uterine tube; which (oedema) predisposes the victim to susceptibility to non-specific infection; HIV infection inclusive (Amadi et al, 2007. Moreover the hormone has been reported to be crucial for optimal immune function (Souba and Smith 1996).

The study thus aimed to look at the thyroid hormone status being a possible primary factor that aggravates the disease process in HIV/AIDS. The justification for this arose from the fact that a number of theories have been put forward in attempt to explain variations in the development of clinical AIDS after an initial HIV infection. These theories include concurrent infection with viruses like cytomegalo virus (CMV) and hepatitis B-virus infection. This study attempts to examine a hormonal state (hypo-/hyperthyroidism) as a factor that may be linked to the development of clinical AIDS with HIV infection. The questions that arise from this are whether HIV infection can affect thyroid hormone formation and/or release? If these were so could it have been through direct influence on the gland

and/or a primary compromise of its function or through another secondary factor? In contrast, could it be that the association between the hypo/hyperthyroid state and HIV/AIDS was that of Hypo/Hyperthyroidism predisposing the individual to clinical AIDS?

Material and methods

The study was carried out on two hundred AIDS patients aged between 20-40 years a sexually active age bracket presenting at a leading AIDS Clinic (FAITH ALIVE) in Jos. The informed consent of the patients and subjects was obtained. They were very enthusiastic because of their anxiety to seeing if our research findings could lead to a cure for their predicament. Fifty (50) volunteer healthy subjects who tested negative for HIV or AIDS were used as controls. They were matched age to age, duration of detected infection and gender groups. All the male control and patient groups were circumcised in early infancy. Thyroid stimulating Hormone (TSH) and free plasma thyroxine (T_4) Total protein, Albumin Globulin. Immune Complex or Complement Fixation and Bence Jones proteins were assayed.

Preparation and examination of blood smear

Venous blood samples obtained under aseptic conditions were processed for microscopy. Thin blood films made from the buffy coat layer and from packed red cells respectively of the samples anticoagulated in 3.8% Sodium citrate of the two groups ie seropositive patients and seronegative controls were prepared. The buffy coat contains Lymphocytes also known as CD-4 cells or thymocytes (T cells). (Souba and Smith, 1996). They were fixed in absolute methyl alcohol for 3 minutes. One volume of Giemsa stain solution was diluted with one volume of phosphate buffer solution (pH 7.0). The slide was flooded and the stain, allowed to stand for 20 minutes. It was then washed and differentiated with the phosphate buffer solution: drained and dried in air at room temperature. The smears were examined for red blood cells, and CD-4 cells count by the longitudinal and battlement methods of Baker, *et al*, 1994).

Comparison of immune complex levels

Immune complex levels and thus complement fixation ability of the two groups were determined by the polyethylene Glycol (PEG) precipitation according to the method of Haskova et al (1978). Each time blood was collected, loss of Immune complex by cryroprecipitation was avoided by allowing the serum to clot at room temperature and determining the immune complex immediately; and the blood was collected in a siliconized or plastic test tube, allowed to clot over one hour, before separating the serum in a centrifuge. To 2ml of buffered PEG 6000, 4.166% solution was added 0.22ml of serum prediluted 1:3 with borate buffer. This was properly mixed to bring the final concentration of PEG to 3.75% and that of serum to1:30. After mixing and incubation for 60 mins at room temperature, the light absorbance of each test solution was read at 450nm against a Blank prepared by adding 0.22ml of prediluted serum to 2ml of borate buffer, mixed and incubated over the same period. Its light absorbance at 450nm wavelength was measured photometrically using 1cm cuvette in a spectrophotometer SP6-2000 (PYE UNICAM). Low concentrations of PEG (3.7%) were used to precipitate soluble antigen-antibody complexes while free antigens and antibodies remain in solution.

Standard immune complex calibration curve (fig. 5)

Aggregated human gammaglobulin (AHG) was used as an in-vitro model (standard) of human immune complexes. The AHG was prepared by heating native human globulin 1.6% (SEVAC. Czechslovakia) in a water bath at 36^0c for 30 mins (Scrimshaw *et al* 1968; Theofilopoulos *et al*, 1976). Concentrations of the standard were prepared in mg/ml: 80, 40, 20, 10, 5, 2.5. To each were added 2.0ml PEG; of standard prediluted 1/3, incubated at Room temperature for 1hr and the turbidity measured at 450nmλ using 1cm cuvette.

Total serum protein estimation

The biuret method of Reinhiold et al (1969) was employed. To 0.1ml of test serum was added 2.9ml of normal saline and 3.0ml of Biuret working solution. For the blank, 3ml of Biuret reagent was added to 3.0ml of normal saline. The tubes were incubated at 37^0c in a water bath for 10 minutes; after which the concentrations were read in the spectrophotometer at 540nm and estimated from a standard protein calibration curve. The serum blanks were read against Tartarate – iodide solutions (Rochelle Salt) and the tests and standard against the biuret blank.

Estimation of albumin

To $4cm^3$ of Bromocresol green (BCG) in a test tube was added $0.02cm^3$ of test serum and mixed. The mixture was allowed to stand for 30mins. It was then read in the spectrophotometer at 640nm. A standard solution containing 3.1g/100ml was similarly treated. The albumin contents of the sera were calculated.

Serum globulins

The differences between the total protein and serum albumin was calculated to give total globulins (Reinhold, 1969).

Bradshaw's test for bence jones proteins (bjp)
One ml of urine was layered gently on 4mls of concentrated hydrochloric acid in a test tube; seropositive and seronegative specimens alike.
Determination of TSH (μ/ML)
Thyroid stimulating Hormone (TSH) was assessed using the double antibody RIA procedure of Surks, *et al* (1972) as specified by WHO International Laboratories (Kits) for Biological Standard London; courtesy of Chemical Pathology UCH, Ibadan. The kits were purchased locally from NUMS Diagnostics which is a subsidiary of UNIS Medical Laboratory Diagnostia Centre Ltd. Ameritek USA.
Determination of free thyroixine (t4) (ng/dl)
The free thyroxine content of all sera was determined as applied by Samuels al (1979) using RIA reagent Kits. WHO International Laboratories; also purchased from Nums Diagnostics.
Statistical analysis
Statistical comparisons for unpaired data were made by applying the student's t-test for mean, standard deviation and standard error of mean on an AMAX computer (Microsoft programme). A probability value of less than 0.05 was regarded as statistically significant.

Results
Table 1 is a summary of result obtained. Seropositives showed very high thyrotropin levels (TSH) but lower thyroxine (T_4) at ($P<0.001$) and higher globulin concentration ($P<0.05$) more than seronegatives Immune complex (C_3) was significantly ($P<0.001$) higher in seronegative males than females. However in females the seropositives had higher immune complex than seronegatives at the same level of significance. Bence Jones Proteins (BJP) was absent (-ve) in seronegatives but positive (+ve) in seropositives.
Blood picture
Figures 2, 3 & 4 are blood smears of the study groups. The red blood cells of seronegative controls were robust and fully haemoglobinized (figure 2). The Lymphocytes of seropositive-clinically AIDS patients appears hollow (Ghosts), with a mean count of $102\pm2.8mm^3$. The nuclei were split into lobules and eccentrically marginalized (Figure 3). The nuclei and cell membrane of cD_{-4} cells of seronegative controls (figure 4) were intact, and the mean count was $10{,}000\pm4mm^3$.

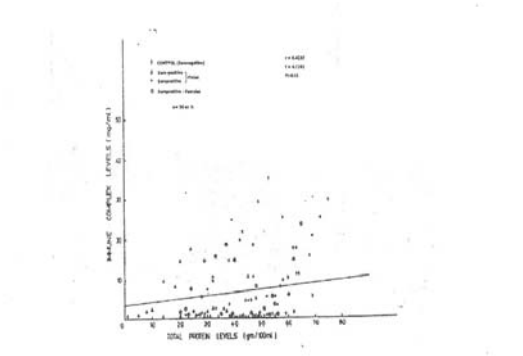

Fig. 1: Correlation between soluble immune complex levels (mg/ml) and serum total protein levels (g.100ml) in seronegative and seropositive HIV/AIDS

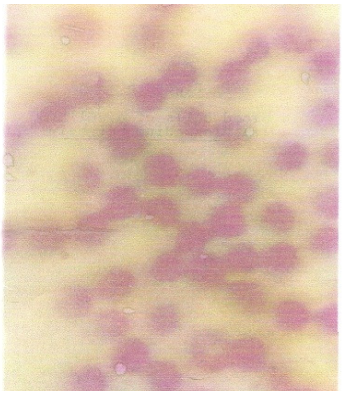

Fig.2: Blood picture of seronegative control. Giemsa stain. Red blood cells appear robust and fully haemoglobinized.

Fig. 3: Thymus lymphocytes of seropositive HIV/AIDS patient. Giemsa stain. The lymphocytes appear hollow (ghost cells). Nuclei of the blood corpuscles split into lobules eccentrically marginalized.

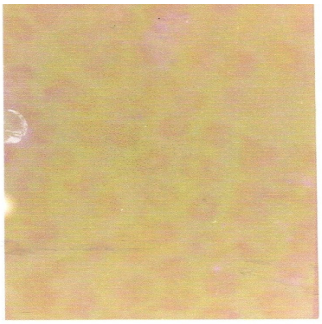

Fig. 4: Thromocytes of seronegative control. The nuclei and cell membrane are intact (Giemsa stain).

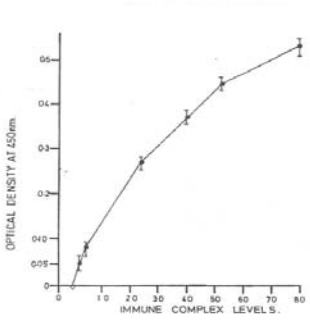

Fig. 5: Immune complex aggregating gamma globulin standard curve

Discussion

Fontes *et al* (2003) reported differences in mineralocorticoid or thyroid function among groups of HIV patients. Ketsamathi et al (2006) reported hypothyroidism in some Thai HIV – Infected patients and found a few of their studied population with subclinical hypothyroidism, another fraction had concurrent low T4 and low or normal TSH, while a few others had overt hyperthyroidism and one with subclinical hypothyroidism. However we found consistent hypothyroidism in all the seropositives studied; as typified by high thyrotropin (TSH) and low thyroxine (T_4) levels. Overt hypothyroidism has been defined as a high TSH and low T4 levels; subclinical hypothyroidism as a high TSH level (Betran et al, 2006).

It has been reported that patients who cannot make their own thyroid hormone cannot compensate for the reduced thyroid hormone levels and hence their thyrotropic hormone level (Judith and Harold, 2001). It would thus appear that the immune system is in a state of regulatory chaos and therefore cannot keep the host alive and healthy probably due to reduced sensitivity to thyroid hormone or the reduced level.

The significant depletion of T4 in HIV/AIDS patients suggest that hypothyroid state predisposes the host cells to nonspecific infection (Amadi et al 2007); HIV inclusive; by the mechanism of transdifferentiation or "Identity deception". This could sustain a different cell line progressively depleted of thyroid hormone and vulnerable to viral assault. Annarosa et al (2005) had reported stem cell transdifferentiation in the adult organism, which is capable of generating mature cells beyond their own tissue boundaries; although they did not explain the causative factor for this phenomenon.

From the distortion of T-lymphocyte (figure 3) in hypothyroid seropositives, the hypothyroid state could be the pedestal for easy transdifferentiation as a result of "stem cell deception" from normal euthyroid cells to immunodeficient cell readily susceptible to viral insult. Identify deception has been documented as a non-crime for stem cells (Judith and Harold, 2001) Proper levels of thyroxine are reported crucial for optimal immune function (Souba and Smith, 1996) but hypothyroid state gives rise to a generation of cells resulting in tissues with loose stroma and oedema (Amadi et al, 2007).

Blood specimens showed depressed mean cD_{4-} cell population (102±2.8mm-3) for seropositive clinically AIDS patients; but normal count of the same cells (10,000±8.4mm-3) for seronegatives (Table 1), which might be an evidence of a gross immune suppression in the patients. The T-lymphocytes of the seropositives portray deplected "ghost cells" (Fig. 3), which appeared polynuclear; depicting hypertrophy of the thymus gland. Hypertrophy which is compensatory for immunocytes might have lead to "neoimmunocytes" producing immunodeficient T. cell not immunopotent to the host. Alternatively most of the globulins have no antibody activity and were globulins but not gamma (γ) globulins or antibodies. The high globulin γlevels in hypothyroid seropositives (Table 1) might confirm observation by some workers that hyperimmunized mice synthesized 5 to 10 times all classes of immunoglobulins (Fontes et al 2003). This might account for the impaired cell-mediated immunity and are thus immunosuppressed. Low plasma albumin and total protein as observed presently (fig. 1) have been incriminated as responsible for poor lymphocyte transformation; consistent with hypothyroidism and presence of BJP found here in seropositives. Presence of Bence Jones Proteins

(BJP) implies a degree of dedifferentiation. (Judith and Harold, 2001).

Paradoxically, significantly higher immune complex levels were isolated in male seronegative while higher values were recorded in female seropositives than controls at P<0.001 respectively. The low levels suggest that this inactive component is being utilized in the activation pathway faster than it can be synthesized whereas high C_3 level are part of the non-specific acute-phase reaction in tissue damage. Complement plays an important part in body's defence against infections as a mediator of both immune and allergic reactions. Invading organism, after identification by antibodies, are lysed by activated complement; the activation being initiated by the antigen-antibody complexes and bacterial liposaccharides or toxins. The antibody fixes complement on the surface of the invading cells; hence the complement fixation test. The level of the complement would indicate the relationship between its synthesis and utilization in the activation pathway.

Protein including albumins and globulins are indispensable for body defence against infection. Protein depletion or its calorie malnutrition depresses immune responses of the subjects or have other adverse effects (Haskova et al 1978; Scrimshaw et al 1968; Theofilopoulos et al, 1976; Reinhold, 1969). Starvation or under-nutrition impairs immunoglobulin (1g) and serum albumin levels; causes diminished mean absolute concentrations of total proteins, albumin, α_2-globulins and β-globulins but had little or no significant effects on gamma globulin concentrations (Surks et al 1972; Samuel et al 1979).

However Cooper, et al (1974) reported depressed formation of antibodies capable of blocking the cytotoxic activity of sensitized lymphocytes (Judith and Harold, 2001). There is impairment of the antibody formation and immunoglobulin levels (Betran et al, 2006); in HIV/AIDS patients.

Bence Jones proteins (BJP) recorded in this work on HIV/AIDS patients is found in the urine of many patients with malignant immunocytomata. Its presence implies a degree of dediffererntiation of immunocytes (Judith and Harold,2001). The paradox could probably be explained in that either the seropositive might not develop enough antibodies to form immune complexes as found in seronegatives or due to less number of antigenic determinants of the complexes and their diminished ability to recognize and produce specific antibodies against the antigen. The complexes might have density less than 19sedberg unit (19S) proteins. Complexes of density less than 19S proteins fix complements poorly or that the antibodies were oligovalent.

Table 1: Summary of TSH, T4, Protein, albumin and globulin levels in seronegative and seropositive HIV/AIDS patients

Groups	*TSH (μ/ml)*	*T4 (μg/dl)*	*Total protein (g/dl)*	*Albumin (g/dL)*	*Globulin*	*CD4 cells (mm3)*	*IC3 (mg/ml)*
Seronegatives	12.1 ±0.02	8.0 ±0.05	12.0 ±0.001 (n=15)	7.95± 0.001 (n=15)	4.09± 0.005 (n=10)	10,000 ±8.4	*Males* 0.74 ±0.58** (n=58) *Females* 0.24 ±0.34** (n=25)
Seropositive HIV/AIDS patients	30.5 ±2.01**	2.45 ±0.54	9.50 ±0.10** (n=15)	3.65 ± 0.56* (n=15)	5.865 ± 0.586* (n=10)	102 000 ± 2.8	*Males* 0.03 ± 0.02 (n=15) *Females* 1.53 ± 0.744** (n=25)

Some workers have shown that the antibodies involved in immune complex formation are normal divalent molecules (Osunkoya, et al., 1972). We have not investigated as yet the sex-difference in complement fixation. Could thyroid hormone replacement and at what optimum dose and period, reverse the decline of immune complex, total protein, albumin, globulin and BJP levels in HIV/AIDS patients? The findings in the present investigation suggest that among other factors, thyroid hormone deficiency (hypothyroidism) could be a primary culprit in seropositive HIV/AIDS disease and that hypothyroid state appears to be a marker to initiate the onset of HIV/AIDS.

Acknowledgement

We acknowledge with thanks the invaluable contributions of Mr. Godwin Njoku of Biomedical illustration and Teaching Unit JUTH for the photomicrographs; Messers S.O. Odunuga and S.O. Adeniran of Immunology Department, University College Hospital (UCH) Ibadan for analyzing the samples. We thank in no small measure Miss Funmilayo Raji for intelligently typing the scripts.

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