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Original Article

Plasma Interferon-gamma and IL-4, Immunoglobulin Classes and Nitric Oxide in Nigerians with Acute Leukaemia

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ABSTRACT

Acute leukaemia are usually rapidly progressive with death often occurring in a few weeks to a few months in untreated patients as a result of abnormal hematopoietic function as well as impaired immune response. The risk of relapse which remains in 20% of patients in remission calls for more research on acute leukaemia. This study therefore, evaluated the plasma levels of nitric oxide (NO), interleukin-4 (IL-4), interferon-gamma (IFN-γ) and immunoglobulin classes (IgA, IgG, IgM, IgE) in twenty-five (25) patients with acute leukaemia (AL) and twenty-five (25) apparently healthy controls. The mean levels of plasma IgA, IgG and IgM were not significantly elevated in leukaemia patients compared with control. However, the mean plasma levels of IgE, NO, IL-4 and IFN-γ were significantly elevated in leukaemia patients compared with controls. It could therefore be concluded from this study that humoural immunity is not depressed in acute leukaemia patients.

Keywords: Clinicopathological, Haematopoietic, Immunosuppresion, Leukaemia, Neoplasm

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INTRODUCTION

Leukaemia is malignant neoplasm that is primarily bone marrow and peripheral blood-based. Stage of differentiation and clinico-pathological characteristics classify leukaemia into different classes such as myeloid, lymphoblastic, lymphoid, acute and chronic. In 2000, approximately 256,000 children and adults around the world developed some form of leukaemia, and 209,000 died from it (Mathers *et al*., 2001). Acute leukaemia (AL) may result in loss of normal hematopoietic function of the bone marrow, with development of anaemia, thrombocytopenia, and granulocytopenia.

Development of malignancies such as leukaemia indicates a dysregulation in the immune system (McClain, 1996) and hematologic malignancies have been shown to be one of the important causes of secondary immunodeficiency (Rosenblatt, 1996). Hyperactive immune response and immune inactivity have both been emphasised in the pathogenesis of acute leukaemia (Narita *et al*., 2001). There has been conflicting reports on quantitative and qualitative immunologic variables in malignancies especially; serum immunoglobulin levels (Potapnev *et al*., 2004). Reports showed that there was no significant difference in mean values of serum immunoglobulin classes in patients with acute lymphoblastic leukaemia (ALL) compared with age matched healthy subjects (Haraldsson *et al*., 1994).

Nitric oxide (NO) is a free radical molecule which is produced in macrophages, neutrophils and lymphocytes (Brunne *et al*., 1997). Significant activity of inducible nitric oxide synthase (NOS) has been reported in tumour cells, including acute and chronic leukemic cells (Brandao *et al*., 2001). Cytokines are released in response to a diverse range of cellular stresses that profoundly affect several stages of cancer formation, growth of tumours *in vivo*, progression and playing a

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significant role in immuno-surveillance against malignant cells (Colombo and Trinchieri, 2002). In acute leukaemia patients, cytokines could be produced both by leukemic and normal cells (Cloppenborg *et al*., 2005; Wu *et al*., 2005). Cytokines have been reported to be important regulators of blast proliferation, but the responses to cytokines have been variable (Bruserud, 1998), therefore the roles of cytokines in the pathogenesis of acute leukaemia is unclear.

IFN-γ is reported to influence many biological processes along with the production of NO_2^- and iNOS in certain tumours (Alexandrova *et al*., 2001). Ghosh *et al*. (2004) observed about three to four fold increase in IFN- γ level patients with acute lymphoblastic patients. Interleukin-4 (IL-4) is produced within the bone marrow microenvironment either by non-resident circulating cells, namely T lymphocytes, mast cells, and basophils or by bone marrow stromal cells (Cardoso *et al*., 2009). Importantly, IL-4 induces proliferation of T-cell acute lymphoblastic leukaemia (T-ALL) cells (Barata *et al*., 2004). The exact mechanisms by which IL-4 induces leukaemia expansion remain unknown but Cardoso *et al*. (2009) suggested that inhibition of IL-4 signalling may have therapeutic potential in T-cell leukaemia since it mediates the proliferation of T-cell acute lymphoblastic leukaemia cells through mTOR-dependent regulation of cell cycle progression.

Although current treatment regimen cures almost 80% of cases, the risk of relapse in 20% of patients is a great concern. Therefore, there is a need to further elucidate the disease biology in acute leukaemia so as to provide information for the first time on the concept of immune status in acute leukaemia patients in Nigeria. To achieve this, we determined plasma levels of innate humoural factors (IFN-gamma, IL-4 and NO) and adaptive humoural factors (Ig G, A, M, E) in a cohort of leukaemia patients.

MATERIALS AND METHODS

Patients

The design is a case control study. Twenty five (25) patients with acute leukaemia [14 Acute Lymphoblastic Leukaemia (ALL) and 11 Acute Myeloblastic Leukaemia (AML)], with the age range of 4 to 50 years were recruited for this study from the Haematology Outpatient Unit of the University College Hospital, Ibadan, Nigeria between March, 2008 and November, 2010. The patients were taking no anti-leukemic therapy and were newly diagnosed cases. Twenty five age-matched healthy subjects were taken as control group. Informed consent was obtained from each participant and the study was approved by the University of Ibadan/University College Hospital (UI/UCH) Joint Ethical Review Committee.

Biochemical Measurements

Five millilitres (5ml) of venous blood were collected into heparinised bottles to obtain plasma after centrifugation for 5 minutes at 4000 x g. The plasma obtained was stored at -20^{0}C until analysed.

Determination of Immunoglobulin Classes (IgG, IgM and IgA)

Immunoglobulin G, A, and M were determined using single radial immunodiffusion as previously described (Arinola *et al*., 2006). The diameter of precipitin ring formed after antigen-antibody reaction in a buffered agar gel is proportional to the concentration of each parameter present in the plasma. Briefly, appropriately measured volume of diluted mono-specific antiserum, specific for each parameter, was properly mixed with noble agar and poured on glass plate. Wells of equal diameter were made in the antibody/agar gel and filled with standards (25%, 50%, 100%, and 200%) or test. The plates were incubated for 4 hours (IgG) and 18 hours (IgA and IgM) at room temperature and the diameters of precipitin rings were measured using an illuminated Hyland viewer with a micrometre eyepiece.

Determination of IgE

Enzyme linked Immunosorbent Assay (MICRO-ELISA, Leinco Technologies, Inc. USA) was used in determining the levels of IgE in the plasma (Barbee *et al*., 1981). The assay system utilises two unique antibodies (a mouse monoclonal and a goat polyclonal) directed against distinct antigenic determinants on the IgE molecule. Undiluted test samples/controls containing IgE were added to plastic wells coated with anti-IgE (mouse monoclonal) to form immune complexes. Anti-IgE (goat polyclonal) enzyme-labelled with horseradish peroxidase was added to each well and incubated for 45 minutes at room temperature. Enzyme chromogen and stopper solutions were added to the wells at room temperature. The intensity of the colour is directly proportional to the concentration of IgE in the sample. The optical density (OD) was read at 450nm wavelength and the OD of samples was used to extrapolate the concentration of IgE

from the standard curve plotted with OD versus standard IgE concentrations.

Determination of Nitric Oxide

Nitric oxide was measured spectrophotometrically as the stable metabolite nitrite (NO_2^-) according to the Griess method (Green *et al*., 1982). Griess reagent (1% sulphanilamide and 0.1% naphthylethylenediamine dihydrochloride in 2% phosphoric acid) was mixed 1:1 with samples. Nitrite formed a coloured chromophore with the reagent which was measured with an ELISA microplate reader at 543 nm. The ELISA kit (G2930) was supplied by Promega, USA.

Determination of IL-4 and IFN-γ

Plasma levels of IL-4 and IFN-γ were determined using ELISA following manufacturer's instruction (MICRO-ELISA, Leinco Technologies, Inc. USA).

Statistical Analysis

Statistical Package for the Social Sciences for Windows (SPSS, version 15.0) was used to analyse results expressed as mean ± SD. Student t-test was used to compare the means of test and control subjects.

RESULTS

Table 1 shows that the mean levels of IgE, NO, IL-4 and IFN-gamma were significantly raised in Nigerian patients with acute leukaemia compared with controls. The levels of IgA, IgG and IgM were not significantly different in the patients compared with the controls.

Table 1: Levels (mean ± SD) of IgA, IgG, IgM, IgE, NO, IL-4 and IFN-γ in Leukaemia Patients and Controls

Parameters (unit of measurement)	Leukaemia (n = 25)	Control (n = 25)	t-values	*p*-values
IgA (g/L)	2.13 ± 2.40	2.50 ± 2.63	0.512.	0.611
IgG (g/L)	14.48 ± 7.56	12.26 ± 6.38	1.126	0.266
IgM (g/L)	1.38 ± 0.64	1.56 ± 0.63	0.984	0.330
IgE (µg/L)	0.31± 0.03	0.29 ± 0.02	2.731	*0.009
NO (µM/L)	33.12 ± 19.61	15.96 ± 10.69	3.841	*0.000
IL-4 (pg/ml)	5.9 ± 0.4	2.7 ± 0.2	2.380	*0.020
IFN-γ(pg/ml)	15.0 ± 9.0	9.0 ± 3.0	3.060	*0.004

P is significant at p < 0.05 value (2-tailed).

DISCUSSION

Acute leukaemia is a progressive clonal disorder that is driven by mutations (Lichtman and Liesveld, 2001). Hyperactive immune response and immune inactivity have been emphasised in the pathogenesis of acute leukaemia (Narita *et al*., 2001). In this study, no significant statistical difference was observed in the plasma levels of IgA, IgG and IgM in leukaemia patients compared with controls. This supports the reports of Okpala and Salimonu (1994) and Mashhadi *et al*. (2009). The normal level of serum IgG at diagnosis was found to be a beneficial prognostic factor associated with lower rate of leukemic cell persistence in peripheral blood and better outcome of childhood B-lineage ALL (Potapnev *et al*., 2004). In an older study, there was depression of both cellular immunity, measured by the number of T cells and skin tests, and humoral immunity, measured by number of B cells, primary antibody production to typhoid vaccine, and levels of immunoglobulin (Guy *et al*., 1976).

In contrast, mean plasma level of IgE was significantly higher in leukaemia patients compared with controls. This contradicts the report of Alsabti (1979) who observed no significant difference in acute myelogenous leukaemia patients. The differences observed could be due to differences in the immunopathological basis of acute lymphoblastic leukaemia and acute myeloid leukaemia since this present study did not separate the patients into acute lymphocytic- or acute myeloid- patients.

Cytokines are released in response to a diverse range of cellular stresses that profoundly affect several stages of cancer formation, growth of tumours *in vivo*, progression and playing a significant role in immunosurveillance against malignant cells (Colombo and Trinchieri, 2002). Significant elevation of plasma levels of NO, IFN-γ and IL-4 were observed in leukaemia patients compared with controls. The observed elevated level of IFN-γ corroborates the report of Ghosh *et al*.

(2004) but contradicts the report of Park *et al*. (2006). The observed elevation could be due to the presence of ligands for 9-O-AcSAa2-6GalNAc glycotope in acute leukaemia patients' serum which is involved in regulating the functional level of IFN-γ (Ghosh *et al*., 2004). More so, cellular stresses associated with leukaemia could induce IFN-γ production especially as IFN-γ is associated with lineage commitment and differentiation of the leukemic cells (Cloppenborg *et al*., 2005; Wu *et al*., 2005).

IL-4 is a growth factor that displays multiple biological activities which depends on cell target and its activation state. It has been reported to possess divergent effects on leukemic progenitors (Manabe *et al*., 1994). Elevated level of IL-4 observed in this study supports the report of Park *et al*. (2006). This elevation might be a result of its antitumor effects and ALL cells growth suppression (Rossi *et al*., 2002). A similar observation was reported in chronic lymphocytic Leukaemia patients by Levesque *et al*. (2006). IL-4 exerts its suppressive activity by blocking the production of autocrine or paracrine growth promoting cytokines, which cooperate in the growth of neoplastic cells *in vitro*, as well as by direct inhibition on the growth of neoplastic cells.

Although nitric oxide has cytotoxic and cytostatic properties in the tumoricidal activity of the immune system, studies have indicated that NO can be an important mediator of tumour growth (Jenkins *et al*., 1995). Elevated plasma level of NO was observed in this study. This is in line with report of Ghaffari *et al*. (2005). Tanaka *et al*. (2002) also reported a similar observation in NO related substrates in some haematological malignancies. The observed elevation could be due to the raised level of IFN-γ since IFN-γ influences the production of NO_2- and iNOS in certain tumours (Alexandrova *et al*., 2001). Additionally, elevated level of IL-4, in conjunction with IFN-γ could also be responsible for the elevated NO since both was reported to induce iNOS expression in leukemic cells (Levesque *et al*., 2003).

Patients with acute leukaemia are immunosuppressed, and this plays a major role in increasing their morbidity and mortality (Hersh *et al*., 1971). Also, immunosuppression is an obstacle to the treatment because immune mechanisms are thought to be important for the control of minimal residual disease (Serody *et al*., 1997). In the present study, the levels of immunoglobulin classes (IgG, A and M) and cytokine (interferon-gamma and interleukin-4) are not reduced in acute leukaemia patients, therefore the concept of immune suppression in acute leukaemia patients might have been caused by other factors apart from reduced IgG, IgA, IgM, interferon-gamma or interleukin-4.

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