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ORIGINAL PAPER

Haemocytometric profile and plasma levels of selected cytokines in patients at various stages of cervical cancer

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ABSTRACT

Introduction and aim. Reports have shown that there is alteration in haematological and inflammatory processes in patients with cervical cancer. However, there is the dearth of information on the pattern of alteration in Nigerian patients with cervical cancer at various stages of the disease. Therefore, haemocytometric profile and plasma levels of interleukin-6 (IL-6) and IL-12 were determined in Nigerian patients with cervical cancer at various stages of the disease.

Material and methods. Eighty-nine adults consisting of 49 patients with cervical cancer and 40 apparently healthy controls were enrolled into this study. Haemocytometric profile was determined using automated haematology analyzer while the plasma levels of interleukin-6 (IL-6) and IL-12 were determined using ELISA.

Results. Of the participants with cervical cancer, 6.12%, 24.49%, 53.06% and 16.33% were in stages I, II, III and IV respectively. The mean plasma IL-6 level was significantly higher in patients at stage IV of the cancer compared with those in stages I, II and III. No significant differences were observed in the mean plasma IL-12 level, and the haemocytometric profile when patients in different stages of the cancer were compared with one another. Plasma IL-6 had significant positive correlation with the lymphocytes count and cancer stage but had significant negative correlation with packed cell volume (PCV), haemoglobin and total white blood cells count (WBC) in patients with cervical cancer.

Conclusion. Interleukin-6 appears to play an important role in the progression of cervical cancer and could be involved in cervical cancer-associated alteration in haemocytometric profile.

Keywords. cancer stage, cervical cancer, haemocytometry, interleukin-6, parity

Introduction

Cervical cancer is the fourth most common cancer in women and the fourth leading cause of cancer death in women globally.[1,2] Although cervical cancer is more common in elderly women, reports have shown that the proportion of young women with the disease has risen from 10 to 40% in the last three decades.[3]

In 2020, an estimated 604,000 new cases and 342,000 cervical cancer-related deaths were reported. Unfortunately, about 90% of the new cases and deaths occurred in poor resource countries.[4] In Nigeria, about 53.3 million women are estimated to be at risk of developing cervical cancer.[5,6] This disproportionately high incidence of cervical cancer in poor resource countries

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has been attributed to myriads of factors including poverty, poor access to healthcare, limited availability of human papilloma virus (HPV) screening service, and limited access to preventative measures. Similarly, late presentation and limited access to quality healthcare are key factors responsible for the high incidence of cervical cancer-related mortalities in the developing countries.[2,7]

Significant alteration in the haemocytometric profile of cervical cancer patients has been reported.[8-10] The report of Hamad[8] showed that cervical cancer patients had elevated leukocyte count, and decreased haemoglobin level and haematocrit compared with healthy women. Similarly, Tavares-Murta et al.[9] reported that leukocytosis, lymphopenia, neutrophilia, and elevated neutrophil-lymphocyte ratio (NLR) were more frequent at advanced stages of cervical cancer compared with the preinvasive neoplasia stage and early-stage. They also suggested that neutrophilia could be a good indicator of cancer invasiveness. In addition, Kose et al.[11] showed that there is a correlation between invasion of cervical cancer and NLR as well as platelet-lymphocyte ratio (PLR).

Antitumor response by the immune system is dependent on production of cytokines that promote a Th1 cytotoxic response.[12] These cytokines mediate proinflammatory activities which are vital in antineoplastic processes. Reports have shown that serum levels of IL-6 are elevated in lung, colorectal, breast, brain, liver, and gynecological cancers.[13-15] Similarly, the reports of Tjiong et al.[16] and Tavares-Murta et al.[17] showed that IL-6 level is elevated in the cervicovaginal washings and in the serum of patients with intraepithelial neoplasia and cancer of the cervix, and that the elevation is associated with invasive cervical cancer and metastasis. It was also shown in an experimental study that there is a significant correlation between local cervico-uterine and serum levels of IL-6 with increasing grades of cervical intraepithelial neoplasia (CIN) and metastasis.[18]

Interleukin-12 (IL-12), a 74kDa heterodimeric glycoprotein, is a potent pro-inflammatory cytokine with antitumor properties.[19,20] Its antitumor activities are mediated by induction of Th1 cell differentiation, increased cytotoxic activities of T, NK and NKT cells, and reprogramming of immunosuppressive cells.[20-25] Reports have shown that IL-12 heightens the production of IFN-γ, a cytostatic, cytotoxic and anti-angiogenic cytokine which can upregulate MHC I and II expression on tumour cells thereby enhancing recognition and lysis of the cells.[26-30] These antitumour activities of IL-12 have been explored in various preclinical and clinical studies where the results showed notable antitumor effects of IL-12 against various malignancies.[24, 31-35]

Aim

Although reports have shown that there is alteration in haematological and inflammatory processes in cancer patients, information on the pattern of alteration in cervical cancer patients at various stages of the disease is sparse in Nigeria. This study was thus designed to determine the haemocytometric profile and serum levels of selected cytokines; IL-6 and IL-12 in Nigerian women with cervical cancer.

Material and methods

Ethics approval

Ethical approval was obtained from the University of Ibadan/University College Hospital (UI/UCH) Joint Ethics Review Committee (UI/EC/17/0019) before the commencement of the study. Also, written informed consent was obtained from each study participant after detailed explanation on the purpose and significance of the study.

Study participants

Ninety-two women consisting of 49 histologically confirmed cervical patients and 49 age-matched apparently healthy women, who served as controls, were enrolled into this study. The patients were enrolled from the Radiation Oncology Clinic, University College Hospital, Ibadan. The controls were randomly selected amongst women who came for routine pap smear test at the Department of Obstetrics and Gynaecology, University College Hospital, Ibadan.

Patients with history of other malignancies and those who were critically ill were excluded from the study. Also, women who have had hysterectomy with the removal of the cervix were not included among the controls.

Data collection

Clinical history and information on demography and risk factors for cervical cancer were obtained using a semi-structured questionnaire.

Blood sample collection

Venous blood sample (10 mL) was aseptically obtained from each study participant; 5 mL each of the blood sample was dispensed into EDTA-containing bottle and lithium heparin containing bottle for haematology and biochemical analysis respectively. Blood samples dispensed into the lithium heparin containing bottles were centrifuged and plasma samples obtained were stored at -20°C until analysed.

Laboratory analyses

Haemocytometric profile was determined using an automated haemocytometer (URIT: 5160E-01262, China) while the plasma levels of IL-6 and IL-12 were determined using ELISA kits following the manufacturer's

instructions (Invitrogen, USA). The immunoplate was read at 450 nm using an Absorbance Microplate Reader (SpectraMax® Plus384). Analytical sensitivity of the kit for IL-6 was <1 pg/mL while that of IL-12 was 0.2 pg/mL.

Statistical analysis

Data were analysed using the Statistical Package for Social Science SPSS (IBM, Armonk, NY, USA) software, version 21.0. Two group mean comparisons were carried out using the Student's t-test for parametric variables whereas; non-parametric variables were compared using the Mann-Whitney U test. Analysis of variance (ANOVA) was used for comparison of more than two groups for parametric variables while Kruskal Wallis test was used for non-parametric variables. Correlations were tested using Spearman rank correlation. Statistical significance was set at $p<0.05$. Results are presented as mean±standard deviation (SD) when parametric and median (interquartile range) when non-parametric.

Results

The distribution of the cancer stages of the cervical cancer patients is shown in Figure 1. More than half of the patients (53.06%) were in stage III while only a few of the patients were in stage I (6.12%) of the cancer (Fig. 1). Histologically, 97.96% of the patients had SCC while 2.04% had AD. NO patient had histological report indicating ADCC.

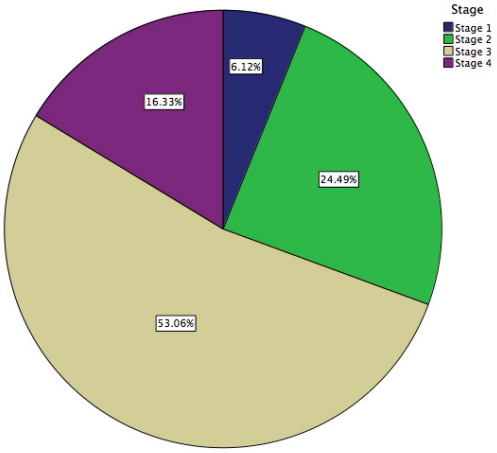

Fig. 1. Distribution of cancer stages in cases

In Table 1, selected characteristics of the study participants, haemocytometric profile, and plasma levels of the cytokines are shown. There was no significant difference in the mean age of patients with cervical cancer compared with the controls. However, parity was significantly higher in patients with cervical cancer compared with the controls.

The mean PCV, haemoglobin (Hb), total white blood cell count (WBC) and platelet count were significantly lower while parity and the mean plasma IL-6 level were significantly higher in patients with cervical cancer compared with the controls (Table 1). The mean neutrophil count, lymphocyte count and serum IL-12 level in patients with cervical cancer and the controls were not significantly different (Table 1).

Table 1. Age, haemocytometric profile and plasma cytokine levels in patients with cervical cancer and controls[a]

Variables	Cases (n=49)	Controls (n=40)	p
Age (years)	52.94±10.64	50.55±1.89	0.321
Parity	5.22±2.06	3.25±1.19	<0.001*
PCV (%)	35.02±3.87	39.63±2.96	<0.001*
Haemoglobin (g/dL)	11.41±1.32	13.22±1.31	<0.001*
WBC (cells/μL)	5126.53±1286.43	6445.0±1031.04	<0.001*
Neutrophil (%)	54.51±6.98	55.98±6.18	0.303
Lymphocytes (%)	42.55±7.87	40.93±5.24	0.266
Platelet (cells/μL)	172571±24298	256673±33738	<0.001*
IL-6 (pg/mL)	18.74 (13.12–61.31)	2.18 (1.79–2.86)	0.001*
IL-12 (pg/mL)	10.08 (6.36–21.61)	11.43 (7.53–16.73)	0.102

[a] * – significant at $p<0.05$; PCV – packed cell volume; WBC – white blood cells count; IL – interleukin

As shown in Table 2, there was significant increase in parity of the cancer patients with increasing stage of the cancer. The mean plasma IL-6 level was significantly higher in patients at stage IV of the cancer compared with those in stages I, II and III. No significant differences were observed in the mean plasma level of IL-12, PCV, haemoglobin, WBC, neutrophil, lymphocyte, and platelet counts when patients in different stages of the cancer were compared with one another (Table 2).

Table 2. Parity, haemocytometric profile and plasma levels of cytokines in patients with cervical cancer at various cancer stages[a]

Variables	Stage I (n=3)	Stage II (n=12)	Stage III (n=26)	Stage IV (n=8)	p
Parity	3.33±1.20	4.75±0.25[a]	5.15±0.40[a,b]	6.88±0.93[a,b,c]	0.035[#]
PCV (%)	32.67±1.45	35.17±1.31	35.31±0.75	34.75±1.26	0.738
Haemoglobin (g/dL)	10.90±0.49	11.29±0.38	11.62±0.30	11.09±0.22	0.654
WBC (cells/μL)	4900±100	5067±325	5169±296	5162±380	0.986
Neutrophil (%)	53.33±0.88	53.75±2.18	55.08±1.54	54.25±1.67	0.942
Lymphocytes (%)	47.0±1.0	40.75±3.14	42.62±1.41	43.38±2.19	0.656
Platelet (cells/μL)	164666±7859	176417±8982	163375±6946	172571±3471	0.6
IL-6 (pg/mL)	28.19 (21.28–29.73)	21.20 (12.71–29.52)	16.30 (12.58–41.51)	197.27 (13.13–559.48)[a,b,c]	0.037[#]
IL-12 (pg/mL)	8.70 (5.80–11.42)	20.15 (7.41–30.21)	8.13 (6.16–18.87)	12.36 (5.62–29.96)	0.777

[*] [#] – significant at $p<0.05$; [a] – compared with stage I; [b] – compared with stage II; [c] – compared with stage III; PCV – packed cell volume; WBC – white blood cells count; IL – interleukin

As shown in Table 3, the plasma IL-6 level had significant positive correlation with the plasma IL-12 level, lymphocytes count and cancer stage but had significant negative correlation with PCV, haemoglobin and WBC in patients with cervical cancer (Table 3). In the controls, the plasma IL-6 level had significant positive correlation with neutrophil count but a significant negative correlation with lymphocytes count. Also, IL-12 had significant positive correlation with PCV, haemoglobin and platelets count (Table 3).

Table 3. Correlation between the cytokine levels, haematological indices and cancer stages in patients with cervical cancer and controls[a]

Correlating pair		Cases (R, p)	Controls (R, p)
IL-6	PCV	-0.307, 0.032*	-0.243, 0.131
	Haemoglobin	-0.296, 0.039*	-0.298, 0.062
	WBC	-0.314, 0.028*	0.145, 0.372
	Neutrophil	-0.227, 0.117	0.327, 0.040*
	Lymphocytes	0.302, 0.035*	-0.446, 0.004*
	Platelet	-0.207, 0.153	0.265, 0.1113
	Cancer Stage	0.372, 0.009*	
IL-12	IL-6	0.587, <0.001*	0.252, 0.116
	PCV	-0.070, 0.635	0.444, 0.004*
	Haemoglobin	-0.068, 0.640	0.417, 0.007*
	WBC	-0.069, 0.639	-0.320, 0.044
	Neutrophil	-0.183, 0.209	0.279, 0.082
	Lymphocytes	0.049, 0.740	-0.094, 0.564
	Platelet	-0.041, 0.777	0.531, <0.001*
	Cancer Stage	0.096, 0.513	

[a] * – significant at $p<0.05$

Discussion

The continuous rise in the proportion of patients with cervical cancer especially, in young women, is of public health concern.[3] This disproportionate high incidence highlights the need for proper understanding of the dynamic changes in haemocytometric profile and cytokines levels during the course of the disease with a view to improving on patients' management.

Iron deficiency and tumour-associated bleeding are common causes of anaemia in cervical cancer.[36] Anaemia seen in cervical cancer has the characteristics of anaemia of chronic disorder associated with low PCV. In this study, the mean PCV and haemoglobin count were significantly lower in patients with cervical cancer compared with the controls. This observation corroborates previous reports which showed that haemoglobin concentration was significantly lower in cervical cancer patients compared with healthy controls.[10, 37-41] Our observation could be due to several factors including poor nutrition due to anorexia associated with cancers generally, haemorrhage, metastasis to the bone marrow thereby causing suppression of erythropoiesis and tumour-associated infections.[42]

Reports have shown that tumour-related leukocytosis (TRL) occurs in 1% to 10% of patients with non-haematopoietic malignancies.[43] This has been attributed to upregulation in haematological growth factors expression. Previous reports have shown that WBC and platelet counts are elevated in cervical cancer patients compared to healthy controls.[8,10,41] Observation from our study was in contrast to these previous reports as WBC and platelet counts were observed to be significantly lower in patients with cervical cancer compared with the controls. The observed lower WBC count in the patients with cervical cancer may be an indication of bone marrow involvement leading to suppressed haematopoiesis, decreased production of haematological growth factors or increased rate of WBC lysis.

Platelets have been reported to play a multifaceted role in cancer progression and metastasis through complex interactions between the platelets and tumour cells resulting in tumour growth, aberrant angiogenesis, invasion, and metastasis.[44,45] The report of Seretis et al. suggested that a normal platelet count could conceal the presence of highly hypercoagulative and pro-inflammatory cancer phenotypes in the presence of efficient compensatory mechanisms.[46] The observed reduction in platelet count in patients with cervical cancer, in this study, may suggest highly hypercoagulative and pro-inflammatory cancer phenotypes with an inefficient compensatory mechanism. This observation may be associated with the cancer stage as most of the study participants with cervical cancer were at stages III and IV.

Alteration in cytokines levels has been associated with most tumours and this alteration is suggested to play a role in cell transformation, cancer cell proliferation, survival, invasivity and metastasis.[47] In this study, the plasma level of IL-6 was significantly higher in patients with cervical cancer compared with the controls. This observation supports previous studies which reported cancer related inflammation with a corresponding increase in circulatory levels of IL-6 in patients with cervical cancer.[48-50] Similarly, studies have shown that there is elevated IL-6 level in vaginal fluid of cervical cancer patients.[51,52] Although IL-6 is a pleotropic cytokine that can either function as a pro-inflammatory or anti-inflammatory cytokine, its potential to promote inflammation in cancer has been demonstrated. Previous studies suggested that IL-6 and its soluble receptor (sIL-6Ra) appear to have a key role in the transition from an acute to a sustained or even chronic inflammation by decreasing neutrophil and favouring mononuclear-cell accumulation.[53-55] During cancer development, an initial acute inflammatory response usually entails neutrophils infiltration of tumour site, but a more sustained population of mononuclear cells later replaces the neutrophils in a process orchestrated by the release of IL-6.[53] Through its soluble receptor (sIL-6Ra), IL-6 activates endothelial cells to produce monocyte chemotac-

tic protein-1, thus stimulating monocyte recruitment,[55] while inducing polymorphonuclear-cell apoptosis.[55,56] This results in attraction of macrophages toward the tumour where they are integrated and thus, represent the major inflammatory component of the stroma as tissue-associated macrophages (TAMs).[57] The TAMs predominantly comprise an M2 population which promotes angiogenesis, tissue remodelling and repair that propagate cancer progression.[58] The study of Wei et al.[59] showed that IL-6 promotes in vivo tumour growth of human cervical cancer C33A cells. Therefore, our observed elevation in IL-6 levels in patients with cervical cancer could be indicative of chronic inflammation which tends to further promote tumour growth and angiogenesis. Therefore, plasma IL-6 level could serve as a diagnostic marker of cervical cancer, however further studies are required to assess its diagnostic performance and its inhibition as a possible immunotherapeutic strategy.

Late presentation by cancer patients is usually associated with high morbidity and mortality. In this study, more than 50% of the patients with cervical cancer were in stage IV of the disease. This observation underscores the need for increased advocacy on voluntary screening for cervical cancer with the view to enhancing early diagnosis which ultimately results in reduced morbidity and mortality.

The observed higher level of IL-6 in patients presenting at late stages (stages III and IV) compared with patients presenting at early stages (stages I and II) corroborates the report of Song et al.[60] which showed that expression of IL-6 is associated with progression and prognosis of human cervical cancer. In the study, IL-6 expression was assessed in cervical cancer tissues histologically obtained. This represents a highly invasive procedure that would be difficult to implement for routine examination. Hence, our observed significant differences in the plasma IL-6 levels between patients at the late and early stages of cervical cancer as well as a positive correlation between plasma IL-6 level and stage of cervical cancer shows promise of a more convenient prognostic marker of cervical cancer that can be implemented for routine monitoring of the course of cervical cancer.

The observed significant negative correlation between the plasma IL-6 level and PCV, haemoglobin and WBC suggests that plasma IL-6 suppresses the process of haematopoiesis. This further confirms our earlier observed lower WBC count in the patients with cervical cancer. Akchurin et al.[61] reported that IL-6 contributes to development of anaemia via induction of hypoferremia, aggravation of renal fibrosis, and alteration of the erythropoietin axis in juvenile chronic kidney disease.

Significant positive correlation between IL-6 levels and stage of gastric cancer as well as colorectal cancer has been reported.[62,63] Similar result was observed in this study as the plasma IL-6 level had significant positive correlation with the cancer stage in patients with cervical cancer. This observation also confirms our observed higher level of IL-6 in patients presenting with stages III and IV cervical cancer compared with patients presenting with stages I and II cervical cancer. These observations indicate that the plasma level of IL-6 increases as the cancer stage progresses.

An association between high parity and risk of cervical cancer has been reported.[64] This has been attributed to high rate of pregnancy-associated cervical abnormalities, increased rate of HPV infection and local changes to cervical cells due to birth-associated traumas.[65] In this study, parity was significantly higher in patients with cervical cancer compared with the controls. Our observation together with the previous reports indicate that there is the need for improved understanding of the interplay between high parity and the risk of developing cervical cancer. This could further enhance our knowledge on cervical cancer preventive strategies.

Conclusion

In conclusion, alteration in haemocytometric profile is a prominent clinical feature in patients with cervical cancer. Also, plasma IL-6 level appears to play an important role in alteration of the haemocytometric profile and the progression of disease in patients with cervical cancer. Therefore, IL-6 could be further explored as an immunotherapeutic intervention in patients with cervical cancer. However, the mechanisms through which parity increases the risk of cervical cancer require further studies.

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Declarations

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Author contributions

Conceptualization, M.A.J., A.A.O. and F.M.A.; Methodology, M.A.J., A.A.O., F.M.A., V.F.E., S.K.R., and O.G.A.; Investigation, M.A.J., A.A.O., F.M.A., V.F.E., S.K.R., and O.G.A.; Data Curation, M.A.J., V.F.E. and S.K.R.; Data Analysis, V.F.E. and S.K.R.; Writing – Original Draft Preparation, M.A.J., A.A.O., F.M.A., V.F.E., S.K.R., and O.G.A.; Writing – Review & Editing, M.A.J., A.A.O., F.M.A., V.F.E., S.K.R., and O.G.A.; Supervision, M.A.J., A.A.O and F.M.A.,

Conflicts of interest

The authors have no competing interest to declare.

Data availability

Data are available upon request from the correspondence author.

Ethics approval

Ethical approval was obtained from the University of Ibadan/University College Hospital (UI/UCH) Joint Ethics Review Committee (UI/EC/17/0019).

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