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ASSESSING THE UTILITY OF A CYTOKINE IN THE DIAGNOSIS OF CERVICAL INTRAEPITHELIAL NEOPLASM AMONG WOMEN IN BENIN CITY, NIGERIA

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ABSTRACT

C ervical cancer has been recognized as the fourth commonest cancer worldwide with 70 % of deaths from cases occurring from developing countries. The mortality rate among women in Nigeria can be drastically reduced by early specific diagnosis of premalignant lesions. Although Cytokines are not routinely used as diagnostic markers for cervical cancer, this study investigated the strength of Granulocyte Monocyte Colony Stimulating growth Factor (GM-CSF) as an indicator in the prediction of Cervical Intraepithelial Neoplasm (CIN). The study was carried out at the Department of Obstetrics and Gynaecology, University of Benin Teaching Hospital Benin City and Department of Chemical Pathology, Edo University Iyamho, Edo state Nigeria, between August, 2017 and January 2019. It was a cross-sectional prospective study. A total of 197 female participants were recruited for this study. Based on histological diagnosis they were grouped as; Negative, CIN 1, CIN 2 and CIN 3. Venous blood was obtained from participants and serum GM-CSF levels were determined using standardized laboratory methods. The results showed a statistically significant elevation of serum GM-CSF in participants with CIN lesions (56.27 ± 1.21ng/ml) compared to those without (33.43 ± 3.03ng/ml). The study also suggests a 56.3% sensitivity and a 95.3% specificity of GM-CSF marker to CIN with an Area under the Curve (AUC) of 0 .907, $p<0.001$ from the Receivers Operator Curve (ROC). This research suggests the use of GM-CSF as a good marker for screening of those at risk of cervical cancer because of its high prospect to reduce the incidence of false positives. However, because of the low sensitivity, it is recommended that GM-CSF be used as a CIN diagnostic tool in combination with the standard Pap smear test.

Keywords: *Benin City, Cervix, Cancer, Diagnosis, GM-CSF, Neoplasia, Smoking, Metabolic medicine*

INTRODUCTION

The prevalence of invasive cervical cancer in Sub-Sahara Africa is on the increase and it is the most common cancer in women globally (Pisani *et al*., 1999). The rising concern and disturbance about cervical cancer is that all women are possibly at risk of developing cervical cancer at some point in their lifetime (Lawicki *et al*., 2008). Cervical cancer is a type of tumor that affects the lowest part of the uterus. A woman is at risk once she turns fifteen. Approximately 80% of cases occur in developing countries (Parkin *et al*., 2005). It is one of the leading causes of death among the female population in Nigeria. In 2007, it was stated that 36.59 million women aged ≥15 years in Nigeria are at risk of developing cervical cancer (Ahmed *et al*., 2013). Luckily, this cancer is avertable by screening for premalignant lesions. Sadly, it is seldom provided and exploited (Gorelik *et al*., 2005).

In developing countries, there is a dearth of active screening tools for premalignant lesions. In Nigeria, no clinically substantial decrease in the incidence of cervical cancer has occurred during the past four decades (Mohammed *et al*., 2006). In advanced countries, by contrast, there has been a major drop in cervical cancer deaths after the institution of extensive cytological testing (Kalliala *et al*., 2005). Diagnostic testing tools for ascertaining cancer ranges from classical methods (Papanicolaou test known as Pap smear) to modern methods. The modern method includes the use of cytokines called hematopoietic growth factors (HGFs). The levels of HGF are used as indicators of possible cancer cells (Averette and Janicek, 2001). The increasing or declining value of indicator levels in prevalent malignancies predict progression or remission of the condition (Lawicki *et al*., 2012).

Investigations to explore the diagnostic potential of hematopoietic growth factors (HGFs) such as (interleukin (IL), interferon (IFN), granulocyte-colony stimulating factor (G-CSF), granulocyte-macrophage-colony stimulating factor (GM-CSF), macrophage-colony stimulating factor (M-CSF), and tumor necrosis factor (TNF) as markers for early diagnosis of Cervical intra epithelial neoplasia (CIN) is critical in the mitigation of this deadly genital cancer. Cytokines play a key role in the regulation of cells of the immune system and also have been implicated in the pathogenesis of malignant diseases (Ahn *et al*., 2005). GM-CSF is a byproduct of the metabolism of tumor cells. This biomarker is the most widely used serum marker for breast cancer. The production of GM-CSF has been demonstrated in a number of non-hematologic malignancies including tumors of the cervix (Watanabe *et al*., 2000). The use of GM-CSF in this study was also due to the hormonal interaction between the cervix and breast of a woman in the reproduction age bracket. This research examined the capacity of GM-CSF as an early indicator in the diagnosis of cervical intra epithelial neoplasia (CIN).

MATERIALS AND METHODS

PARTICIPANTS

A total of 197 participants were recruited for this study at the Urogynaecology and Gynae-oncology Unit of the Department of Obstetrics and Gynaecology, University of Benin Teaching Hospital (UBTH) from August 2017 to January 2019. Informed consent was obtained from participants during study.

METHODOLOGY

The biochemical analysis of the research participants was done at the Metabolic Research Unit of the Department of Chemical Pathology, Edo University Iyamho, Edo state, Nigeria. Cases included patients with histological diagnosis of cervical intraepithelial neoplasm and abnormal Pap smear referred from Gynaecology Clinic, General Practice Clinic, Community Development Clinic in UBTH and also referrals for Colposcopy from private hospitals in Benin City. Control participants were selected from the Gynaecology clinic of UBTH and were patients with normal Colposcopy who matched the case for age and parity. Socio-demographic characteristics of participants in the study consisted of information relating to age, unsuccessful conception, marriage type and smoking habit, which were possible risk factors for CIN.

Venipuncture was done for all the participants for collection of blood sample for biochemical analysis. 5 ml of blood samples was collected into a plain tube, allowed to clot and retract, thereafter centrifuged at 2000 rpm for 15 minutes. The serum was collected with Pasteur pipette into a plain tube and then stored at about – 4 °C until the biochemical analysis was carried out. The biochemical analysis of the granulocyte monocyte colony stimulating growth factor (GM-CSF) was carried out using the enzyme linked Immunosorbent Assay (ELISA) method.

DATA ANALYSIS

Data collected were analyzed using SPSS version 20. Results were presented using Tables, Plates and graphs. Quantitative variables were expressed as mean $\pm$ standard deviation. Pearson's Coefficient of Correlation was used to correlate occurrence of GM-CSF with other risk factors associated with CIN in the participants. Receiver Operator Characteristic (ROC) curves was used for the best-fitting model, whereas Area Under Curve (AUC) was used for prediction of GM-CSF. One-way analysis of variance was used to analyze GM-CSF values for the various CIN statuses.

RESULTS AND DISCUSSIONS

The socio-demographic information of the study participants showed that there was a total of 53 participants that were negative for CIN lesions, compared to 144 that had CIN lesion based on histological diagnosis (Table 1). The participants were also grouped according to age, with most of the participants in both control and CIN groups belonging to the 51 – 60yrs age category. More than 30% of the participants with a positive CIN histological result were smokers, compared to 13.2% in the control. This perhaps may point to smoking as being a risk factor for CIN. Although there were a significant number of smokers within those who were positive for CIN (see Table 1), the distribution of the GM-CSF results in the smokers did not significantly differ from non-smokers. Mean GM-CSF in the smoking group was 47.93 ng/ml, whereas the value was 50.93 ng/ml within the non-smoking group (Fig. 1).

Several human non-hematopoietic cancers have been shown to express increased levels of GM-CSF (Ohwada *et al*., 2006). CIN status obtained from Pap smear results were in three stages; early stage, intermediate and full-blown stage. They were represented as CIN 1, 2 and 3 respectively. Fifty-three women were diagnosed negative to CIN. Those with CIN lesions had a mean GM-CSF of 56.27±1.21ng/ml which was significantly higher than those of the control group (33.43±3.03 ng/ml) (Table 2). This is similar to the findings of Ławicki *et al*. (2008, 2012) who reported

that in a group of cervical cancer patients, the plasma levels of GM-CSF, G-CSF and M-CSF were significantly different compared to the healthy controls. The result in Figure 2 defined the Receiver Operator Characteristic (ROC) curves for standard GM-CSF benchmark and identified the best-fitting model of GM-CSF at 55.3ng/ml. This place the diagnostic strength of the marker at stage 2 of the CIN lesion. The ROC curve is a resourceful way to appraise the predictive capacity of a parameter with continuous values and is used to evaluate the discriminating power of a diagnostic test (Liu *et al*., 2005). In the correlation model presented in Table 3, GM-CSF examined with other risk factors associated with CIN, had significant positive correlation at 0.01 and its resulting ROC curve had high AUC value of (>0.907). However, it was found to be a low sensitive predictor of CIN. This observation from the present study is similar to the report by Lyon and colleagues (Lyon *et al*., 2008) in a study where multiple cytokines were used in the diagnosis of breast cancer. The bulk cytokine model comprising of GM-CSF performed less than the others investigated in terms of sensitivity to the disease.

The diagnostic efficiency of tumor markers like GM-CSF depends on variety of factors such as sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) (Leek and Harris, 2002). The sensitivity of tumor marker is the probability that the test results will be positive if a tumor is present. Based on the result from the coordinates of the ROC curve, GM-CSF marker was not sensitive to CIN at stage 1 and may not be useful as an early marker (Table 4, Plate 1). Plates 2, 3, and 4 also shows sections of dysplastic squamous epithelial cells involving the lower two-thirds, thickness of the ecto cervix, as well as normal squamous epithelial cells lining the ectocervix.

The findings in Table 5 and 6 compares the diagnostic strength of GM-CSF as a marker for predicting CA cervix against histology. GM-CSF confirms negative to CIN as predicted by histology at 92.5%. The Negative Predictive Value (NPV) value was 94. 1 % (Table 5). NPV describes the probability that the disease is not actually present if the test result is negative (Liu *et al*., 2005). This shows the capacity of the biomarker as a high specific predictor of healthy patients. Specificity of tumor marker as a screening parameter indicates whether it may be used for describing the percentage of healthy individuals with negative test results (Campbell, 1994).
It can be deduced from this research that GM-CSF is a low sensitive biomarker to CIN but highly specific in detecting patients without the disease.

The capacity for GM-CSF for diagnosis of CIN was compared with histology (Table 7). Using histology of the pap smear obtained from a total of 197 participants, histology was able to identify 144 participants to be positive for CIN, whereas 53 were negative. However, the use of GM-CSF, based on the cut-off score obtained (see Figure 2, Table 4) divided the participants into 122 individuals with a negative premalignant lesion and 85 participants with positive CIN pap smear. This indicated that GM-CSF was only able to identify 43.15% of the participants to be positive for CIN lesion, compared to the 73.10% identified by pap smear. GM-CSF is therefore not a good marker based on the results of histology.

CONCLUSION

Theoretically, an ideal biochemical marker should be extremely sensitive and specific, have low false positives, high positive and negative predictive value, most essentially should be 100 % accurate in distinguishing between healthy persons and patients with premalignant conditions. This research finds GM-CSF as an ideal marker for arbitrary population screening because of its high prospect to drastically reduce the incidence of false positives. However, it is recommended that GM-CSF be used as a CIN diagnostic tool in combination with the standard Pap smear test.

Table 1: Socio-demographic characteristics of participants in the study

Parameters		CIN group (n=144)		Control group (n=53)		df	X^2	p-value
		(n)	(%)	(n)	(%)			
CIN.Staus	Negative	0	0.0	53	26.9	3.0	0.463	0.925
	Level 1	45	22.8	0	0.0			
	Level 2	49	24.9	0	0.0			
	Level 3	50	25.4	0	0.0			
Age group	31-40 yrs	17	11.8	15	28.3	4.0	5.784	0.216
	41-50 yrs	32	22.2	10	18.9			
	51-60 yrs	56	38.9	20	37.7			
	61-70 yrs	10	6.9	8	15.1			
	>70 yrs	29	20.1	0	0.0			
Educational level	Primary Edu.	58	40.3	5	9.4	2.0	2	0.368
	Secondary Edu.	16	11.1	26	49.1			
	Tertiary Edu.	70	48.6	22	41.5			
Parity	1.00	0	0.0	9	17.0	8.0	11.501	0.175
	2.00	6	4.2	0	0.0			
	3.00	24	16.7	11	20.8			
	4.00	38	26.4	17	32.1			
	5.00	32	22.2	6	11.3			
	6.00	19	13.2	8	15.1			
	7.00	0	0.0	2	3.8			
	8.00	22	15.3	0	0.0			
	11.00	3	2.1	0	0.0			
Unsuccessful conception	None	142	98.6	49	92.5	3.0	4.386	0.223
	Once	0	0.0	4	7.5			
	Twice	1	0.7	0	.0			
	More than twice	1	0.7	0	.0			
Marriage type	Monogamy	127	88.2	37	69.8	1.0	2.4	0.121
	Polygamy	17	11.8	16	30.2			
Smoking habit	Smokers	46	31.9	7	13.2	1.0	2.4	0.121
	Non-Smokers	98	68.1	46	86.8			
History of usage of OCP	No history	66	45.8	24	45.3	4.0	5.564	0.234
	<1 yr	68	47.2	9	17.0			
	1-3 yrs	4	2.8	5	9.4			
	4-6 yrs	0	0.0	12	22.6			
	>6 yrs	6	4.2	3	5.7			

Table 2: GM-CSF values for various CIN status.

CIN Status	(n)	Mean ± SEM (ng/ml)	95% CI			ANOVA	
			Mean CIN	Lower	Upper	F-statistic	p-value
Negative	53	33.43^c ± 3.03	-	27.34	39.51	47.94	<0.001
Stage 1	45	47.89^b ± 1.71		44.46	51.32		
Stage 2	49	51.23^b ± 1.57	*56.27 ± 1.21*	48.08	54.38		
Stage 3	50	68.74^a ± 1.64		65.44	72.04		

Means with similar alphabetic superscripts on the same column do not differ from each other (p>0.05).
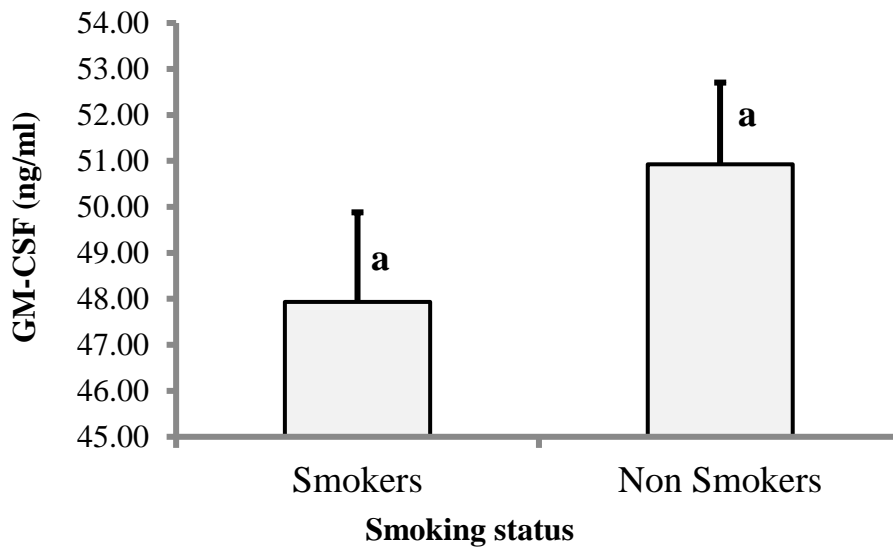

Figure 1: Distribution of GM-CSF values of the study participants based on history of smoking

Table 3: Correlating GM-CSF with other risk factors associated with CIN.

Parameters	CIN.Staus	GM-CSF
CIN.Staus	1	0.637**
Age	0.336**	0.075
Age group	0.259**	0.062
Pariity	0.204**	-0.066
Unsuccessful Conception	-0.086	-0.058
Smoking	0.018	0.068
History of OCP	-0.035	0.055
GM-CSF	0.637**	1

Values presented are Pearson's Coefficient of Correlation
*. Correlation is significant at the 0.05 level (2-tailed).
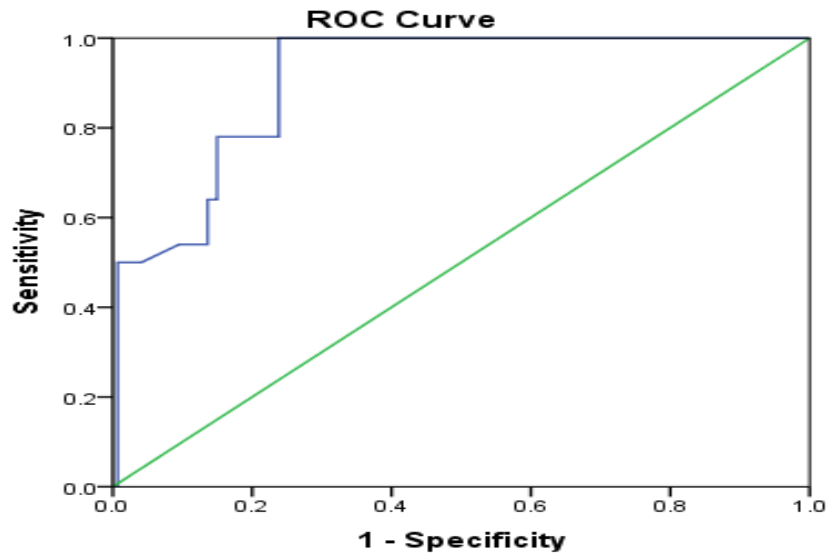**. Correlation is significant at the 0.01 level (2-tailed).

Figure 2: ROC for diagnostic statistical presentation of GM-CSF as a possible biomarker for CA cervix (AUC =0.907, p<0.001).

Table 4: Coordinates of the receiver operator characteristic (ROC) Curve

Positive if Greater Than or Equal To[a]	Sensitivity	1 - Specificity
10.00	1.00	1.00
36.35	1.00	0.71
38.20	1.00	0.64
41.00	1.00	0.60
42.15	1.00	0.58
46.10	1.00	0.39
47.60	1.00	0.35
49.35	1.00	0.32
51.15	1.00	0.29
52.80	1.00	0.27
53.50	1.00	0.26
54.50	1.00	0.25
55.30***	*1.00*	*0.24*
55.65	0.78	0.24
82.50	0.14	0.01
87.50	0.12	0.01
123.80	0.00	0.01
160.60	0.00	0.00

The test result variable(s): GMCSF has at least one tie between the positive actual state group and the negative actual state group.

a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Table 5: 2x2 contingency for CA cervix prediction by GM-CSF vs. histology

Parameters			By GM-CSF		
			Normal	CIN	Total
Histology	Normal	Count	49	4	53
		% within Histology	92.5	7.5	100.0
	CIN	Count	63	81	144
		% within Histology	43.8	56.3	100.0
Total		Count	112	112	85
		% within Histology	56.9	56.9	43.1

Table 6: Sensitivity and specificity of GM-CSF as a marker for predicting CA cervix

Parameters	Value	p-value
Sensitivity	56.30%	-
Specificity	95.3%	-
PPV	95.3	-
NPV	43.8	-
Prevalence of disease	43.1	-
Pearson Chi-Square	37.46	<0.001
Likelihood Ratio	43.655	<0.001
Linear-by-Linear Association	37.271	<0.001
Cramer's V	0.436	<0.001
Odds Ratio	15.750	-
Relative risk	2.113	-

Table 7: Prediction capacity of GM-CSF compared to histology

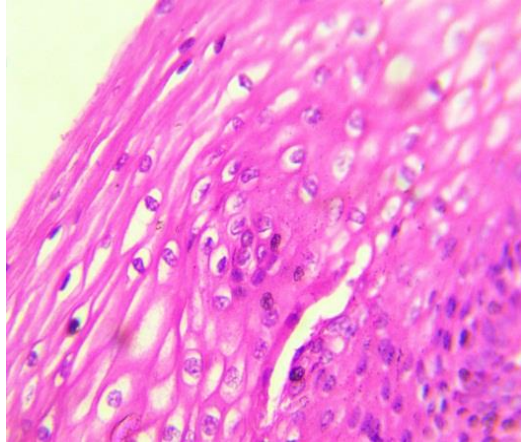| Parameters	Normal	Positive for CIN	Percentage CIN identified	Total
Separation of patients by histology	53	144	73.10	197
Separation of patients by GM-CSF	122	85	43.15	197

Plate 1: CIN-1 x40. Sections showing dysplastic squamous epithelial cells involving the lower third of the ecto cervix.
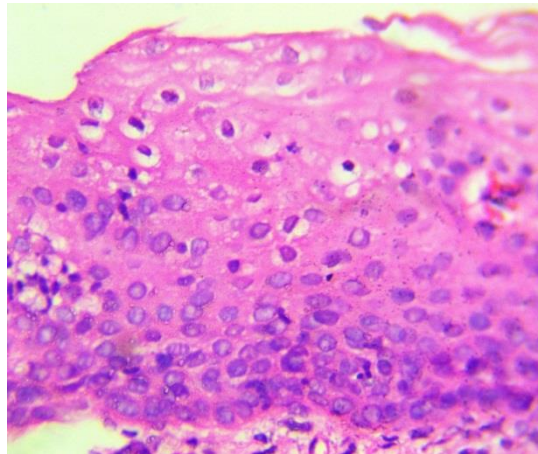

Plate 2: CIN-2 x40. Sections showing dysplastic squamous epithelial cells involving the lower two-thirds of the ecto cervix.
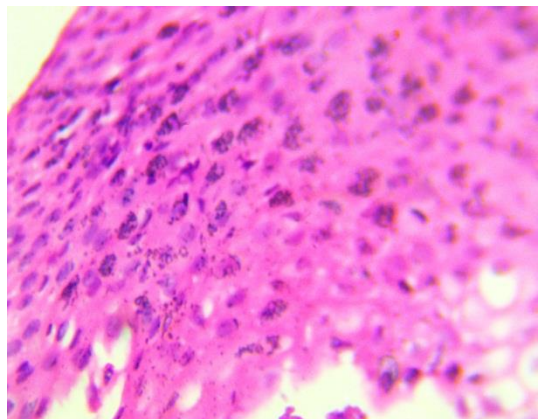

Plate 3: CIN-3 x40. Sections showing dysplastic squamous epithelial cells involving the full thickness of the ecto cervix.
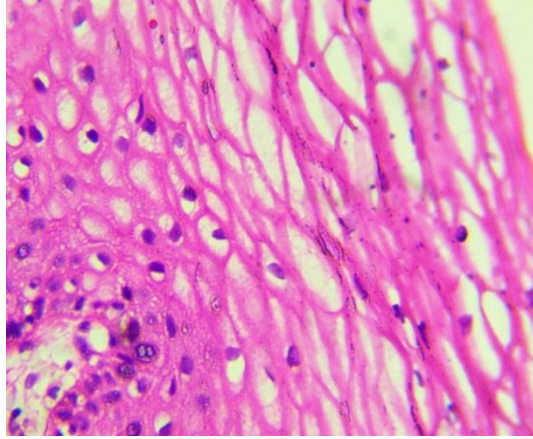
Plate 4: Normal x40. Sections showing normal squamous epithelial cells lining the ectocervix.

REFERENCES

Ahmed, S.A., Sabitu, K., Idris, S.H., Ahmed, R. (2013) Knowledge, attitude and practice of cervical cancer screening among market women in Zaria, Nigeria. *Nigerian Medical Journal*, 54: 316-9

Ahn, H.J., Park, Y.H., Chang, Y.H *et al*. (2005). A case of uterine cervical cancer presenting with granulocytosis. *Korean Journal Internal Medicine,* 20: 247–250.

Averette, H.E, Janicek, M.F. (2001). Cervical cancer prevention, diagnosis and therapeutics. *CA: A Cancer Journal for Clinicians,* 51: 92–114.

Campbell, G. (1994). Advances in statistical methodology for the evaluation of diagnostic and laboratory tests. *Statistics in Medicine* 13(5–7) : 499–508.

Gorelik, E., Landsittel, D.P., Marrangoni, A.M., Modugno, F., Velikokhatnaya, L., Winans, M.T., *et al*. (2005). Multiplexed immunobead-based cytokine profiling for early detection of ovarian cancer. *Cancer Epidemiology, Biomarkers & Prevention*, 14(4) : 981–987.

Kalliala, I., Anttila, A., Pukkala, E., Nieminen, P. (2005). Risk of cervical and other cancers after treatment of cervical intraepithelial neoplasia: retrospective cohort study BMJ 2005;331: 1183 doi: https: //doi.org/10.1136/bmj.38663.459039.7C

Lawicki, S., Będkowska, G.E., Gacuta-Szumarska, E., Knavol, P., Szmitkowski, M. (2008). The plasma levels and diagnostics utility of stem cell factor (SCF) and macrophage-colony stimulating factor (M-CSF) in cervical cancer patients. *Polish Medical Journal,* 25: 38–42.

Leek, R., Harris, A. (2002). Tumor-associated macrophages in breast cancer. *Journal of Mammary Gland Biology and Neoplasia,* 7(2): 177–189.

Liu, H., Li, G., Cumberland, W.G., Wu, T. (2005). Testing statistical significance of the area under a receiving operating characteristics curve for repeated measures design with bootstring. *Journal of Data Science*, 3(3): 257–278.

Lyon, D.E, McCain, N.L, Walter, J., Schubert, C. (2008). Cytokine Comparisons Between Women with Breast Cancer and Women with a Negative Breast Biopsy. *Nursing Research*, 57(1): 51–58.

Mohammed, A., Ahmed, S.A., Oluwole, O.P., Avidime, S. (2006). Malignant tumours of the female genital tract in Zaria, Nigeria: Analysis of 513 cases. *Annals of African Medicine,* 93-96.

Ohwada, M., Wada, T., Saga, Y. *et al*. (2006). C-kit overexpression in neuroendocrine small cell carcinoma of the uterine cervix. *European Journal of Gynaecologiocal Oncology*. 27: 53–55.

Parkin, D.M., Bray, F., Ferlay, J., Pisani, P. (2005). Global center statistics, 2002. *CA: A Cancer Journal for Clinicians,* 55: 74–108.

Pisani, P., Parkin, D.M., Bray, F., Ferley, J. (1999). Estimates of the worldwide mortality from 25 cancers in 1990. *Internal Journal of Cancer*, 83: 870-3.

Watanabe, A., Wachi, T., Omi, H. *et al*. (2000). Granulocyte colony-stimulating factor-producing small-cell carcinoma of the uterine cervix report of a case. *Diagonistic Cytopathiology,* 23 : 269–274.