ISSN: 2536-7153 SJMLS

Sokoto Journal of Medical Laboratory Science 2024; 9(2): 268 – 279

SJMLS - 9(2) - 031

Evaluation of CD4+T Cells, Neutrophils, Basophils and Eosinophils in Chemotherapeutic Colorectal Cancer Patients, Attending Oncology Unit, Usmanu Danfodiyo University Teaching Hospital Sokoto

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Abstract

Colorectal cancer is one of the most common types of gastrointestinal cancers in Nigeria. It was once described as rare among Nigerians in the 1960s, but at present, the incidence of colorectal cancer is rapidly growing. The occurrence of colorectal cancer in Nigeria has been linked to both environmental and hereditary factors, with environmental factors playing a more significant role, due to economic impact, changes in dietary and lifestyles. The aim of the present study was to evaluate the level of CD4 cells, neutrophils, basophils and eosinophils in chemotherapeutic colorectal cancer patient attending Usmanu Danfodiyo University Teaching Hospital. A total of 57 subjects consisting of 30 chemotherapeutic colorectal cases and 27 healthy controls were recruited in the research. Five (5) mls of venous blood was collected from each patient. The total leukocytes and differential counts were performed as well as the level of CD4+T-cells. The results indicated a statistically significant ($P < 0.005$), increases in the levels of CD4+ T-cells, neutrophils, basophils and eosinophils in chemotherapeutic colorectal cancer (CRC) patients. In the CRC patients on FOLFOX regimens, the CD4+, neutrophils and basophils, were statistically significant at p-value 0.0001, while eosinophils have p-value of 0.0003, but in CRC on FOLFIRI regimen, CD4+, neutrophils were statistically significant at p-value 0.0001,while basophils have P-value 0.0006, and eosinophils have P-value 0.0023. In patients on CapeOX regimens, they was no statistically significancy in all the groups. In conclusion, statistical significance was observed in patients taking FOLFOX and FOLFIRI chemotherapeutic regimens on CD^{4+}, neutrophils, and basophils in comparison with the CapeOX. The eosinophil was not statistically significant.

Keywords: Colorectal cancer, CD4 cells, Neutrophils, Basophils, Eosinophils and Chemotherapy.

Introduction

Colorectal cancer is the most common type of gastrointestinal cancer in Nigeria. It was once described as rare disease amongst Nigerians in the 1960s, but at present, the incidence of colorectal cancer is rapidly growing in Nigeria. The increasing occurrence of colorectal cancer in Nigeria has been linked to both environmental and hereditary factors, with environmental factors playing a more significant role in Nigeria, due to economic development and changes in dietary and lifestyles preferences (Irabor *et al.,* 2010). Colorectal cancer was the 10th most common malignancy among men and women respectively in 1960, and as at 2018, it is the 2nd most common cancer in men and the 3rd most common cancer in women in Nigeria. Due to the poor access to healthcare facilities in Nigeria, most patient with colorectal cancer, present late, at a point where disease cure is usually not achievable. In view of an increasing colorectal cancer incidence in Nigeria, the disease pathogenesis, clinical presentation, histologic subtypes, disease risk factors and modes of treatment are explored in this review, while highlighting changing trends and peculiarities of the disease in Nigeria (Badoe *et al.,* 2009).

As a major treatment modality for many advanced cancers, conventional chemotherapy can achieve high response rates but is rarely curative. The mounting evidence that many chemotherapeutic agents have immunostimulatory effects has provided a compelling rationale for developing combined chemoimmunotherapy strategy to achieve improved patient outcomes (Prendergast and Jaffee, 2007). Current cancer immunotherapies predominantly rely on CD8+ T cells to fight against tumors. Although it is increasingly clear that proinflammatory CD4+ effector T cells are critical determinants of effective antitumor immune responses (Rakhra *et al.,* 2010). Many anticancer drugs can cause varied degree of lymphodepletion. It has been well established that lymphodepletion induced by chemotherapy or radiotherapy profoundly enhances the efficacy of adoptive cell therapy (ACT) and cancer vaccines (Klebanoff *et al..,* 2005). This is likely due to the combined effects of creation of space and increased availability of stimulatory growth factors that lead to enhanced proliferation and survival of activated T cells (Gattinoni *et al..,* 2005). In this regard, cyclophosphamide is a representative anticancer drug that causes profound lymphodepletion while creating an immune milieu rich of type I interferons (IFNs) and common gamma-chain cytokines (IL-2, IL-7, and IL-15) (Moschella *et al..,* 2011). Besides strengthening the activities of immune cells, chemotherapy also promotes the trafficking of activated immune cells to the sites of tumor (Ramakrishnan *et al..,* 2010). Accumulating evidence demonstrates that there is a surge of proinflammatory cytokines/ chemokines, such as GMCSF, IL-1β, IL-6, and CXCL10, in the post-chemotherapy immune milieu, which may contribute to the recruitment and retention of tumor-reactive immune cells, including activated CD8+ and CD4+ T cells, DCs, macrophages, and neutrophiles, in the tumor microenvironment (Schiavoni *et al.,* 2011). A great deal of effort has been focused on understanding how chemotherapy potentiates CD8+ T-cell responses (Hirschhorn-Cymerman *et al.,* 2009). And enhances antigen presentation (Radojcic *et al.,* 2010). Although tumor-reactive CD4+ effector/helper T cells are increasingly recognized as critical determinants of effective antitumor immune responses, the effect of chemotherapy on these cells is largely neglected, and the role of CD4+ T cells in modulating post-chemotherapy host immunity is almost entirely unknown. In the following we mainly focus on findings that concern the impact of chemotherapy on the interactions between tumors and CD4+ T cells. Effect of Chemotherapy on CD4+ T-Cell effectors development. So far, among the aforementioned anticancer drugs, cyclophosphamide (CTX) appears to be the most effective one in enhancing antitumor CD4 responses, particularly when used in combination with adoptive cell therapy (ACT). It has been demonstrated in various preclinical models that CTX treatment followed by adoptive transfer of tumourreactiveCD4+ T cells, either monoclonal T-cell clones derived from TCR-transgenic mice or activated polyclonal CD4+ T cells derived from pre-immunized mice, leads to eradication of established tumors (Ding *et al.,* 2010).

Standard cytotoxic chemotherapy can initially achieve high response rates, but relapses often occur in patients and represent a severe clinical problem. Some of the most common possible side effects include; hair loss and nail changes, mouth sores, loss of appetite or increased appetite, nausea and vomiting, pancytopenia (anaemia, leucopenia, neutropenia and thrombocytopenia), predisposition to infections, easy bruising or bleeding (thrombocytopenia), fatigue (anaemia), menstrual changes, infertility, neuropathy (taxanes, platinum agents, vinorelbine, erubulin, and ixabepilone), cardiomyopathy (doxorubicin and epirubicin), hand-foot syndrome (capecitabine and liposomal doxorubicin), chemo brain (observable decrease in mental functioning), increased risk of leukaemia (myelodysplastic syndrome and acute myeloid leukaemia), (Erhabor, 2016).

In patients with metastases in distal organs, the addition of irinotecan to fluorouracil and leucovorin was demonstrated to prolongs survival in patients and was considered the new first-line standard therapy for this disease in patients with stage 3 cancer receive complementary chemotherapy after surgery for 6 to 8 months, improving symptoms and prolonging survival in people with stage iv cancer primarily (Herbert *et al.,* 2004).

The5-fluoracil continues to be the cytostatic mostly used in the treatment of colon cancer, those who make use of it or at least approach the fulfilment of the therapeutic standard, achieve better percentages of survival. Currently, the role of an antibody variant called bevacizumab

(AVASTIN) approved by the us food and drug administration (FDA) has been studied by lowering vascular endothelial growth factor (VEGF), the main angiogenesis regulator, produced by normal and neoplastic cells 50 preclinical trials have shown that a human monoclonal antibody against VEGF can inhibit the growth of xenografts of human tumors (Ferrara *et al.,* 2003). Despite all these trials, not much is known about the effects of some chemotherapeutic agents such as folfox, folfiri and CapeOX on the immunological cells. So the aim of this research was, the evaluation of immunological cells amongst therapeutic colorectal cancer patients, attending Oncology unit, Usmanu Danfodiyo University Teaching Hospital Sokoto.

Materials and Methods

Study Area

The study was carried out in the Department of Immunology, School of Medical Laboratory Sciences, Usmanu Danfodiyo University Sokoto, in collaboration with Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto. Sokoto has a population of 3.70 million people based on the March 2006 census. The state has a landed area of 26,64km^2 and is located between longitude 11.30 to13.50 degrees east and latitude 4 to 6-degree north. It is bounded to the North by Niger Republic, Kebbi state to the South-west and to the East by Zamfara state. The state comprised of 23 local governments areas.

Inclusion Criteria

All consented adult male and female patients (volunteers) of $\geq$ 18 years and who were histologically diagnosed with colorectal cancer and are on anti-tumour chemotherapeutic agents attending UDUTH, Sokoto, were recruited into the study. While control group were Normal subject Students from College of Health Sciences, Usmanu Danfodiyo University Sokoto.

Exclusion Criteria

All histologically confirmed colorectal cancer patients who are not on the stipulated anti-tumour drugs or are less than <18years, and non-consented patients attending UDUTH, Sokoto, was excluded from this study.

Study design

The research is simple cross-sectional studies.

Determination of sample size

The sample size was calculated using the standard formula for calculating minimum sample size. Sample size (n) is given by the formula below:

$n=Z_{1-a}\backslash 2^2Pq\backslash d^2$

Where

n = Minimum sample size

$Z_{1-a\backslash 2}$= Percentage point of the normal distribution corresponding to the required (two-sided) Significant level (α) of 0.05=1.96 (Awosan, 2017).

There was no any referenced published data which explored a classified prevalence rate on the level of CD4+ T-cells cells, neutrophils, basophils and eosinophils amongst therapeutic colorectal cancer patients, 50% was considered as the prevalence.

P= Prevalence to be estimated is 3.5% = 3.5\100=0.035

Compliment of p will be 1-p = 1-0.035 = 0.965

d= precision or Tolerance margin of error = 90% i.e. (100-95%) = 5% = 0.05

Substituting these values into the above gives,

n= $(1.96)^2$ X 0.03 X 0.965/ $(0.05)^2$ = 0.129775\0.0025

Hence, non-response (attrition) rate of 10% of 52 = 5.2

52+5.2=57.2.

Ethical consideration

Ethical clearance for the study was sought from Health research and ethics committee of Usmanu Danfodiyo University Teaching Hospital (UDUTH HREC), Sokoto in Sokoto metropolis, Sokoto State, UDUTH/HREC/2021 1080/.V2.

Study Population

The study comprised of 57 histologically diagnosed colorectal cancer patients on chemotherapy attending UDUTH, Sokoto, and potential healthy voluntarily individual Students from (Collage of Health Sciences, UDUS) who have no history of cancer.

Data Collection
The socio-demographic data and other relevant information of each participant were obtained using self-administered questionnaire. Subsequently, data was analysed using simple frequency analysis. Informed consent was sought from willing participant through written form.

Blood Collection and Processing
About **5mls** of venous blood was collected from each of the research participants (case and controls). The blood allowed to stand on the working bench for about 5-10 minutes before proceeding with the laboratory analysis.

Laboratory Analysis
CD4+ cells Counts
PartecCyflow counter was used for the enumeration of CD4+ cells.in all the samples of blood from the patients and controls.

Total Leukocytes Count
Haematological parameters will be analysed using haematology analyser (Sysmex automated haematology analyser model KX-21N, manufactured by Sysmex Co-operation Kobe, Japan)

Three parts differential haematology analyser was used which consist of neutrophils count, eosinophils, and basophils.

Estimation of differential leukocyte count
A differential white cell count (leukocyte formula) consists of an examination of blood to determine the presence and the number of different types of white blood cells. It is obtained by examining a blood film or a peripheral blood smear. A differential white cell count was performed using a manual method

Statistical Analysis
Quantitative data was analysed by the InVivoStat version 4.2.0 Software program, summary statistic, non-parametric analysis (Mann-Whitney test), comparison of un-pair t-test, EXCEL Microsoft (Ms) was used in data input, Descriptive statistics was employed to illustrate the demographic characteristics of the sample by the IBM SPSS Statistics version 20 Software program in simple frequency statistic.

Results
Socio-demographic and Clinical Characteristic
The socio-demographic and clinical characteristic of the research participants is shown in table 1. About thirty (n= 30) chemotherapeutic colorectal cancer patients (52.63%) and twenty-seven (n= 27) apparently healthy individuals as control (47.37%). About 52.63% (N=30) were married, while 47.37% (n=27) were single. Other demographic characteristics are as shown in table 1.

The Level of CD4+ T-cells, Neutrophils, Basophils and Eosinophils in chemotherapeutic Colorectal Cancer Patients
The Level of CD4+ T-cells, Neutrophils, Basophils and Eosinophils in chemotherapeutic Colorectal Cancer Patients is as depicted in Table 2. The level of CD4+ T-cells, in chemotherapeutic CRC Patients at lower 95% confidence interval (CI) was 850.12, neutrophils (7.89), basophils (1.00) and eosinophils (0.64) respectively. While the Upper 95% CI of the CD4+ T-cells was (1001.55), neutrophils (10.12), basophils (1.40) and eosinophils (1.17). The CD4+ show standard error of mean (SEM) (46.86), neutrophils (0.63), basophils (0.16) and eosinophils (0.04), t-test of chemotherapeutic CRC of CD4 was (25.00), neutrophils (16.51), basophils (10.33) and eosinophils (7.00) at *df* (degree of freedom) = (29); while for control the CD4+ T-cells at Lower 95% CI was (835.81), neutrophils (3.14), basophils (-0.008) and eosinophils (0.20) while the CD4+ at Upper 95% CI (1020.93), neutrophils (3.66), basophils (0.25) and eosinophils (0.41). SEM of CD4+ (45.03), neutrophils (26.69), basophils (1.93) and eosinophils (7.24). T-test of CD4+ (20.62), neutrophils (26.69), basophils (1.93) and eosinophils (7.24).*df* = (26), p-value (0.0001) was statistically significant at < 0.005.

The Effects of Combinations therapy on Immune cells in Colorectal Cancer Patients
The effects of Chemotherapeutic combinations in CRC Patients based on different regimens FOLFOX, FOLFIR and CapeOX is as depicted in table 3a. When the patients are subjected to FOLFOX, the level of CD4+ was (884.87±160.84)*, neutrophils (8.33±3.20)*,

basophils (1.28±0.66)* all was statistically significant at p-value (0.0001)[a] while eosinophils (0.95±0.76)* with p-value (0.0003)[a]. Patients on FOLFIRI, are found to have CD4+ of (887.62±231.22), neutrophils (8.47±3.15) with P-value (0.0001)[a] basophils (1.19±0.93) with P-value (0.0006)[a] and eosinophils (1.07±1.00) with P-value (0.0023)[a]. In the case CapeOX; the patients has CD4+ (729.00±321.03) with p-value $(0.8840)_b$, neutrophils (9.70±0.00) p-value $(0.4226)_b$, basophils (0.70±0.85) with p-value $(0.4574)_b$ and eosinophils (1.10±1.13) with p-value $(0.2989)_b$.

Effects of Chemotherapeutic Regimens on Immune Cells in Gender related Colorectal Cancer Patients

The effects of Chemotherapeutic regimens on the immune cells in gender related colorectal cancer patients is as depicted in table 3b. On gender for females, mean±standard deviation of CD4+ (M±SD) at P-value (0.2857) neutrophils P-value (0.0001), basophils p-value (0.3406) and eosinophils p-value (0.4534). For Male mean±standard deviation of CD4+ (M±SD) at P-value (0.0001), neutrophils p-value (0.0001), basophils p-value (0.0001) and eosinophils p-value (0.4534).

Effects of Chemotherapeutic Regimens on Immune Cells in Age related Colorectal Cancer Patients

The effects of chemotherapeutic regimens on immune cells in different aged group in CRC patients is as depicted in table 4. In the age 25-35, CD4+ (p-value=0.5701), and neutrophils (p-value=0.7276) is statistically insignificant, basophils (p-value =0.0399) and eosinophils (p-value= 0.0402) is statistically significance at p-value is < 0.005. Between the ages 36-45 the p-value of the CD4+, neutrophils, basophils, and eosinophils is statistically insignificance> 0.005. While the age between 46-55 was statistically insignificance> 0.005. As well as the age between 57-70 is statistically significance at p-value of neutrophils (p-value =0.0348).

Table 1: Socio-Demographic and Clinical Characteristics

Group	Number of subjects (n)	Percentage
Colorectal patients	30	52.63
Controls	27	47.37
	57	**100**
Marital Status		
Single	30	52.63
Married	27	27
Widow	0	0
Divorced	0	0
	57	**100**
Gender		
Male	38	66.67
Female	19	33.33
Age	**57**	**100**
25-30	13	22.80
31-35	7	12.28
36-40	5	8.77
41-45	3	5.26
46-50	5	8.77
51-55	5	8.77
56-60	5	8.77
61-65	2	3.51
65-70	13	22.81
	57	100

Occupation		
Business	6	10.52
Civil servant	12	21.05
Students	27	47.37
Retired	3	5.26
House/ wife	9	15.78
	57	100
Location		
Sokoto	14	24.56
Kebbi	10	17.54
UDUTHS	27	47.37
Zamfara	5	8.77
Others	1	1.75
	57	100
Tribe		
Hausa	47	82.46
Others	10	17.54
	57	100
Control Chemotherapy FOLFOX		
YES	15	26..32
NO	42	73.68
	57	100
FOLFIRI		
YES	13	22.81
NO	44	77.19
	57	100
CapeOX		
YES	2	3.51
NO	55	56.14
	57	100

Table 2: Level of CD4+ T-cells, Neu, Baso, and Eoso in Chemotherapeutic CRC Patients and Controls

Parameters	Chemotherapeutic CRC					Control					p-value	RM
	Lower 95%CI	Upper 95%CI	SEM	*df*	t-test	Lower 95%CI	Upper 95%CI	SEM	*df*	t-test		
CD4 (cells/µl)	850.12	1001.55	46.86	29	25.00	835.81	1020.93	45.03	26	20.62	0.0001	**SS**
Neutr (cells/*l*)	7.89	10.12	0.63	29	16.51	3.14	3.66	0.13	26	26.69	0.0001	**SS**
Baso (cells/*l*)	1.00	1.40	0.16	29	10.33	-0.008	0.25	0.06	26	1.93	0.0001	**SS**
Eosin (cells/*l*)	0.64	1.17	0.04	29	7.00	0.2	0.41	0.04	26	7.24	0.0001	**SS**

KEY: CD4+=cluster of differentiation type 4, Neu = Neutrophils, Baso = Basophils, Eos=Eosinophils CI = confidence Interval, SEM =standard error ofmean ,*df*= degree of freedom (n-1), p-value = statistical significant which is ≤ 0.005, SS = statistically significant, RE= Remark

Table: 3a: The Effects of Combinations therapy on Immune cells in Colorectal Cancer Patients

Parameters	FOLFOX	p-value	FOLFIRI	p-value	CapeOX	p-value
CD4+(cells/µ*l*)	884.87±160.84*	0.0001[a]	887.62±231.22	0.0001[a]	729.00±321.03	0.8840[b]
Neu (cells/*l*)	8.33±3.20*	0.0001[a]	8.47±3.15	0.0001[a]	9.70±0.00	0.4226[b]
Bas (cells/*l*)	1.28±0.66*	0.0001[a]	1.19±0.93	0.0006[a]	0.70±0.85	0.4574[b]
Eos (cells/*l*)	0.95±0.76*	0.0003[a]	1.07±1.00	0.0023[a]	1.10±1.13	0.2989[b]

KEY: CD4+ = Cluster of differentiation T-cells type 4, Neu= Neutrophils, Baso= Basophils, Eos= Eosinophils, FOLFOX=5 Fluorouracil + Leucovorin +oxaliplatin, FOLFIRI=5Fluorouracil + leucovorin + irinotecan, CapeOX=Capecitabine + Oxaliplatin. The superscript [(a)] is statistically significant as ≤ 0.005, while subscript$_{(b)}$ is non-statistically significant as ≥ 0.00.

Table 3b: Effects of Chemotherapeutic Regimens on Immune Cells in Gender related Colorectal Cancer Patients

PARAMETERS	FOLFOX	FOLFIRI	CapeOX	P-value	REMARK
CD4+ (cells/µl)	1015.00 ± 56.78 ♀	943.33 ± 227.26 ♀	792.00 ± 86.27 ♀	0.2857	NS
	920.00 ± 215.41 ♂	934.33 ± 167.67 ♂	603.50± 143.54♂	0.0001	SS
Neu (cells/*l*)	9.33 ± 3.40 ♀	8.27± 3.87 ♀	8.20 ± 4.24 ♀	0.0001	SS
	9.50 ± 2.90[+]♂	10.12 ± 2.13 ♂	10.05 ± 0.40 ♂	0.0001	SS
Bas (cells/*l*)	1.27 ± 0.12*♀	1.30 ± 0.17 ♀	1.05 ± 0.07 ♀	0.3406	NS
	1.23 ± 0.97[+]♂	1.40 ± 0.24 ♂	0.90 ± 1.13 ♂	0.0001	SS
Eos (cells/*l*)	0.60 ± 0.61 ♀	1.27 ± 0.21 ♀	0.45 ± 0.21 ♀	0.4534	NS
	0.95 ± 0.95[+]♂	0.98 ± 0.66 ♂	1.90 ± 00.00 ♂	0.0001	SS

Key: Mean ± standard deviation*♀ (female colorectal patients), Mean ± standard deviation[+]♂ (male colorectal Patients), FOLFOX=5Fluorouracil+Leucovorin+oxaliplatin, FOLFIRI=5Fluorouracil + leucovorin + irinotecan, CapeOX= Capecitabine + Oxaliplatin, SS= statistically significant, NS= Non-statistically significant.

Table 4.: Effects of Chemotherapeutic Regimens on Immune Cells in various Age Group of Colorectal Cancer Patients

AGE	PARAMETERS	FOLFOX	FOLFIRI	CapeOX	P-value	REMARK
25-35						
	CD4 (cells/µ*l*)	878.00	928.57	789.50	0.5701	NS
	Neu (cells/l)	9.73	9.49	8.60	0.7276	NS
	Bas (cells/l)	0.56	1.37	0.15	0.0399	SS
	Eos (cells/l)	2.07	1.13	2.45	0.0402	SS
36-45						
	CD4 (cells/µ*l*)	1071.33	973.50	792.00	0.0547	NS
	Neu (cells/l)	11.00	12.70	8.20	0.4266	NS
	Bas (cells/l)	2.00	2.00	1.05	0.1539	NS
	Eos (cells/l)	0.27	0.45	0.36	0.2234	NS
46-55						
	CD4 (cells/µ*l*)	901.00	833.00	770.67	0.6369	NS
	Neu (cells/l)	10.167	7.00	7.33	0.3022	NS
	Bas (cells/l)	1.13	1.35	0.80	0.8819	NS
	Eos (cells/l)	0.72	0.85	0.83	0.4384	NS
56-70						
	CD4 (cells/µ*l*)	937.50	968.00	892.00	0.7531	NS
	Neu (cells/l)	4.70	11.25	4.70	0.0348	SS
	Bas (cells/l)	1.35	0.80	1.40	0.5750	NS
	Eos (cells/l)	0.20	0.90	0.30	0.3270	NS

Key: CD4 = Cluster of Differentiation T-cell type 4, Neu = Neutrophils, Bas = Basophils, Eos = Eosinophils, FOLFOX=5Fluorouracil+Leucovorin+oxaliplatin, FOLFIRI=5Fluorouracil+leucovorin+irinotecan, CapeOX=Capecitabine+Oxaliplatin, SS= statistically significant, NS= Non-statistically significant. REMK= Remarks

Discussion

White blood cells are part of the immune system and protect the body against infections and inflammation, and some inflammatory diseases relate to colorectal cancer (Health Europa, 2020). Basophils, eosinophils, and neutrophils are types of white blood cells used to fight allergies, infections, and diseases.

The level of CD4+T-cells, neutrophils, basophils, and eosinophils have statistically significant difference in the mean SEM at 95% CI of each of these components between the chemotherapeutics colorectal cancer patients and controls. Goshen *et al*., (2017) reported a statistically significant difference in mean levels of each of these components between those with and without a diagnosis within 6 months for males and females. Zhou *et al*. (2017) analysed both components between cases and controls using Mann–Whitney U tests. Those diagnosed had a median neutrophil percentage that was higher compared to those without a diagnosis.

The Effects of Combinations therapy on Immune cells in Colorectal Cancer Patients shows the level of CD4+ T-cells based on the FOLFOX is statistically significance at p-value (0.0001) at means standard deviation (SD)884.87±160.84,The means (SD) of

neutrophils (8.33±3.20) at P-value = (0.0001) is statistically, that of basophil is statistically significance p-value = (0.0001) at mean (SD) 1.28±0.66 and eosinophils p-value = (0.0003) mean (SD) of (0.95±0.76).Similar to Madu *et al*., (2013) reported based Pattern of CD4 T - lymphocyte values in Cancer Patients on Cytotoxic Therapy (374.50±189.30) with (t-test p = 0.04), absolute neutrophil count (3.250 ±1.060) with (t-test p=0.17).This is similar to the findings of Khan *et al*. in his study on solid tumours include colorectal cancer patients.

The CD4+ T-cells, neutrophils, based on FOLFIRI was statistically significant at p-value = (0.0001) means (SD) 887.62±231.22, the neutrophils means (SD) of (8.47±3.15) at P-value = (0.0001) is statistically significant, the basophils mean (SD) of (1.19±0.93) p-value = (0.0006), and eosinophils mean (SD) of (1.07±1.00) p-value = (0.0023).

The CD4+ T-cells based on CapeOX was not statistically significant mean (SD) 729.00±321.03 at p-value = (0.8840), neutrophils mean (SD) (9.70±0.00) p-value = (0.4226), basophils mean (SD) (0.70±0.85) P-value = (0.4574), and eosinophils means (SD) (1.10±1.13) P-value = (0.2989). Another important study which has only been presented in abstract form, those treated with capecitabine had higher rates of tumour down staging (52% vs 39%; *P* = 0.16) and increased N0 status (71% vs 56%; *P* = 0.09); however, neither result was statistically significant. As expected, the patients receiving capecitabine had less leucopenia (25% vs 35%; *P* = 0.04). The CAIRO study investigated the benefit of sequential versus combination therapy using capecitabine, oxaliplatin, and irinotecan (Koopman *et al*., 2007). In another study, FOLFIRI was found to be superior to either alternative with a median PFS of 7.6 months compared to 5.9 months form IFL (*P* = 0.004) and 5.8 months for CapeIRI (*P* = 0.015). OS results trended toward superiority at 23.1 months for FOLFIRI, 17.6 months form IFL (*P* = 0.09) (Fuchs *et al*., 2007).

Effects of Chemotherapeutic Regimens on Immune Cells in Gender related Colorectal Cancer Patients was not statistically significant at p-value=0.2857. The male subjects showed a significant difference at (p-value = 0.0001). The difference in neutrophil count of both gender was statistically significant at p-value = 0.0001. The male shows statistically significant difference of basophils at p-value = 0.0001, while the female showed no statistically significant difference in the level basophils P-value=0.3406, eosinophil, female is statistically insignificant at p-value =0.4534, while the eosinophils in male is statistically significance p-value = 0.0001.

Effects of Chemotherapeutic Regimens on Immune Cells in various Age Group of Colorectal Cancer Patients indicated that in the aged group 27-36, CD4+ (p-value=0.5701), and neutrophils (p-value=0.7276) was not statistically significant at p- value is > 0.005, basophils (p-value =0.0399) and eosinophils (p-value= 0.0402) was statistically significant at p- value is < 0.005. Between the ages 37-46, the p-value of the CD4+, neutrophils, basophils, and eosinophils were not statistically significant (p> 0.005). While the age between 47-56 is statistically insignificance (p> 0.005). As well as the age between 57-70 is statistically significance at p- value of neutrophils (p-value =0.0348).

A study also shown age and gender are significant determinants of CD4 count and its percentage. CD4 count and percentage of CD4 were significantly higher in females than in males and declined significantly with increasing age. From the neonatal period to adulthood, the percentage of CD4 shows less variation than that of CD4 count. The World Health Organization has stated that percentage of CD4 rather than the CD4 absolute count may be preferred as the best surrogate marker for monitoring HIV-infected children of all ages, Females significantly had a higher absolute mean CD4 count (1077 ± 609 versus 965 ± 589) and percentage of CD4 (34% ± 6% versus 30% ± 7%) than adults; similarly, children had a significantly higher absolute mean CD4 count. (1770 ± 821 versus 807 ± 260) than the adults, while there was no significant difference in their percentage of CD4 (32%± 0% versus 32% ± 1%) in this study. Other socio-demographic variables had no significant effects on the CD4 count.

CD4 count declined significantly with increasing age (P-value <0.0001). The absolute mean CD4 count of adult males (P- value < 0.001) and females (P- value <0.001) was significantly lower in this study than in the national study (Afolabi *et al*., 2014).

Souglakos *et al*. (2006) the combination of infusion 5-FU/leucovorin, oxaliplatin, and irinotecan (FOLFOX was compared with FOLFIRI in 2 randomized, phase III trials reported no significant differences in OS, TTP, or RR between the 2 treatment regimens.

According to Heinemann *et al*. (2013) that findings of several key studies presented at the 2013 Annual Meeting of the American Society of Clinical Oncology (ASCO) provided important updates to the current picture. In the FIRE-3 (FOLFIRI plus cetuximab versus FOLFIRI plus bevacizumab as first-line treatment for patients with metastatic colorectal cancer) trial, patients with wild type KRAS were randomized to receive first-line FOLFIRI with cetuximab or FOLFIRI with bevacizumab. The primary end points of overall RR (62% vs. 58%, respectively) and PFS (10.0 vs. 10.3 months, respectively) were not significantly different in the 2 treatments arms. However, FOLFIRI with cetuximab provided a statistically significant improvement in OS compared with FOLFIRI with bevacizumab (28.7 vs. 25.0 months, respectively; P ¼ .017). A further important contribution to the on-going first-line therapy debate in mCRC was the TRIBE (Combination Chemotherapy and Bevacizumabas First-line Therapy in Treating Patients with Metastatic Colorectal Cancer) trial, Falcone *et al*. (2013). Bin *et al*. (2011) reported that there were no significant differences in OS and RR between the 2 regimens; however, UFT/leucovor in had a significantly lower toxicity rate than bolus 5-FU/leucovorin (P<0.001) for stomatitis/ mucositis, Grade 1-4 leukopenia, febrile neutropenia, and infection). These findings are consistent with a pooled efficacy analysis from 2 phase III studies that compared capecitabine (another oral 5-FU prodrug) with bolus 5-FU/leucovorin (Van Cutsem *et al*., 2004).

Conclusion

Colorectal cancer affects mostly males aged from 30 years and above. Evaluation of the level of immunological cells, such as CD4+T-cells, neutrophils, basophils, and eosinophils in chemotherapeutic colorectal cancer patients will help in improving the treatment and overall management of colorectal cancer patients.

Recommendations

Chemotherapeutic regimens such as FOLFOX and FOLFIRI may be suitable regimens induce and cause enhancement of antitumor immunity, and thus recommended for cancer patients, especially colorectal cancers. Support programmed from Governments and other non-governmental organisations should be initiated to the less- privileged cancer individuals due to their financial statues. Concerted research should be undertaken and sustained on how immune cells and other chemicals mediators performed in the constant interplay in the elimination of cancer cells among chemotherapeutic colorectal cancer patients.

Conflict of Interest: There is no conflict of interest to declare

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Citation: Alhassan Hussaini Mohammed, Usman Malami Aliyu, Shumsudeen Mohammed , Raji Halima Yunisa and Hamisu Abdullahi. Evaluation Of $Cd4^{+}$T Cells, Neutrophils, Basophils and Eosinophils in Chemotherapeutic Colorectal Cancer Patients, Attending Oncology Unit, Usmanu Danfodiyo University Teaching Hospital Sokoto. ***Sokoto Journal of Medical Laboratory Science*; 9(2): 268 – 279.** https://dx.doi.org/10.4314/sokjmls.v9i2.31