Analysis of Interleukin-6 and Interleukin-8 in a Cohort of Patients with Colorectal Cancers in Sri Lanka

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Abstract*: Colorectal cancer (CRC) is one of the most prevalent cancers globally, accounting for nearly 10% of all cancers. Interleukin-6 (IL-6) and Interleukin-8 (IL-8) levels have been reported to increase in CRC patients. The studies on IL-6 and IL-8 levels of CRCs have confined to Caucasian populations and levels of these cytokines have not been extensively investigated in South Asian populations. They have the potential of using as markers but are not being used in clinical practice, yet. Therefore, the aim of this study was to investigate the serum IL-6 and IL-8 levels in a cohort of Sri Lankan patients. Blood samples from thirty five patients with CRCs and thirty five healthy volunteers were obtained after informed consent. The concentrations of IL-8 and IL-6 were measured using ELISA according to manufacturer's protocols. The mean serum concentration of IL-6 was found to be significantly higher in the CRC patients than controls ($p<0.05$). Although the mean serum concentration of IL-8 was higher in the CRC patients than controls the difference was not significant ($p>0.04$). Interestingly, the mean serum [IL-6] in colorectal cancer patients were correlated with the disease stage. The study provided preliminary evidence to use IL-6 as potential biochemical marker to be used in the diagnosis of CRCs. However, it is necessary analyze more patient samples to validate the results of this study.*

Keywords: *Colorectal cancer, Interleukin-6, Interleukin-8, Serum, Diagnosis*

1. Introduction

Colorectal cancer (CRC) is one of the most prevalent cancers globally, accounting for nearly 10% of all cancers (Sung et al, 2020). It affects over 1.93 million people globally and over 0.9 million deaths have been reported in 2020 (WHO, 2022). There has been a rise in both morbidity and mortality over the years, with predicted rise by about 80% in 2035 (Douaiher*et al*., 2017). Majority of CRCs are sporadic (70 – 80%) (Yamagishi *et al*., 2016) and the remaining are classified as familial (20-25%) (Lichtenstein P, et al 2000) and inherited (5%) (Jasperson et al 2010). Development of CRCs occurs due to alterations of the genetic composition and environmental factors (Rattray *al*., 2017). Inflammation has been identified as a key determinant in CRC pathogenesis and progression (Long *et al*., 2017).

Aberrations in the signalling pathways lead to the abnormal production of cytokines. (Klampfer L., 2011). Cytokines are known to be inflammatory mediators that determine both pro-tumorigenic and anti-tumorigenic signals within the tumour environment (Shrihari TG, 2017). Both systemic and local changes in cytokine profiles have been observed in CRCs (Akhmaltdinova *al*., 2020). Interleukins are types of cytokines, which have been identified to play a role in tumorigenesis angiogenesis, cancer cell invasion, metastasis of CRCs (Pretzsch et al., 2019). Therefore, it is important to study interleukins in CRCs as they play a role in the

development, progression and survival of patients (Park, et al, 2020).

Interleukin-6 (IL-6), a central player in CRCs, is a prototypic inflammatory cytokine (Waldner *et al*., 2012) that is overexpressed in CRC tissues (Nagasaki *al*., 2014) and is known to be involved in the development of sporadic CRCs (Waldner *et al*., 2012). It acts as a growth factor for human CRC cells (Sun et al., 2020). This inflammatory cytokine is secreted by stimulated monocytes, fibroblasts, endothelial cells, macrophages, T-cells and B-lymphocytes (Akira *et al*., 1993). Serum IL-6 levels are elevated in CRCs and correlates with large tumor size, advanced stage, occurrence of liver metastases and reduced survival (Vainer *al*., 2018). Moreover, increased blood IL-6 concentration in CRC patients is an adverse prognostic marker of survival (Shiga *et al*., 2016). DNA mismatch repair defects, angiogenesis (Tseng-Rogenski *et al*., 2015, Wang et al 2020) and accumulation of myeloid-derived suppressor cells in tumors are directly promoted by IL-6, facilitating tumour progression (Lin *et al*., 2020).

Interleukin-8 (IL-8) is an inflammatory cytokine that is mainly produced by macrophages, T cells, B cells and plays a vital role in the inflammatory response of cells (Gonzalez-Aparicio 2022). IL-8 expression is upregulated in tumour tissue of CRC patients (Rubie *et al*., 2007). *In vitro* experiments done on CRC cell lines have shown that IL-8 promotes tumour growth, cell proliferation, metastasis, angiogenesis (Rubie *et al*., 2007) and chemoresistance (Burz *al*., 2021). IL-8 influences the growth and invasion of CRC cells through various mechanisms. An increase in serum IL-8 levels correlates with high tumour grade, increased invasion into the liver, growth and progression of the tumour (Rubie *et al*., 2007), all of which accounts to poor prognosis of CRCs (Xia *et al*., 2015).

Cytokine concentrations in blood have been investigated in studies confined to Caucasian populations. Data on South Asian populations is sparse. Increasing research evidence show that they can be used as markers for CRCs but are not being used in clinical practice, yet. Therefore, it is worth to assess the use of them as biomarkers for CRCs which has not been evaluated adequately in South Asian populations.

The aim of this study was to investigate serum IL-6 and IL-8 levels in a cohort of Sri Lankan patients with benefits of developing potential non-invasive biomarkers which may be useful for the diagnosis, prognosis and efficacious therapeutic approaches for CRCs.

2. Methodology

A. Patient selection

Thirty five patients diagnosed with colorectal cancer reported to the University Hospital of General Sir John Kotelawala Defence University (UHKDU) and National Cancer Institute (NCI), Sri Lanka during the period of January 2021 to December 2021 were recruited for the study after the informed consent. This study was conducted in accordance with the Helsinki Declaration and ethical clearance was obtained from the Ethics Review Committee of the Faculty of Medicine, General Sir John Kotelawala Defence University. Permission was obtained from oncologists and oncological surgeons to recruit their patients. Patients and volunteers were recruited only after written informed consent. Patients who were not able to provide written informed consent, patients under 18 years of age, patients who have undergone treatment for colorectal cancer (surgery/chemotherapy/radiotherapy) and patients with other cancers, chronic infections, HIV, chronic diseases, diabetes, immune disease, cardiovascular and cerebrovascular disease were excluded. The control group consisted of thirty five healthy volunteers, with no

comorbidities and family history of malignancy, who visited the Blood Bank of UHKDU.

B. Samples

Demographic and relevant clinical data were recorded. Whole blood samples (3–5 mL) were collected in plain tubes containing no anticoagulant, and transported to the laboratory at 4 ℃. Samples were obtained at the time of diagnostic/follow up blood sampling and from the same blood donation cannulation in the control group. Serum was separated by centrifugation at 1000xg for 15 minutes in a refrigerated centrifuge and stored at -80 ^{0}C until use. Serum was analysed after histology was confirmed. The samples were stored with a code assigned to it instead of the participant's name.

C. Enzyme-linked immunosorbent assays

All serum samples were removed from –80 ^{0}C and left to thaw on ice at room temperature before analysis. Serum IL-8 (n=35) and IL-6 (n=15) of patients and controls were analyzed using commercial ELISA kits (Elabscience). The assays were performed according to the manufacturer's instructions. All samples were tested in duplicates.

D. Statistical analysis.

T-test was used to investigate if there is a significant difference between serum IL-6 and IL-8 cytokine levels in patients and the control group. *P*-values less than 0.05 were considered to be statistically significant. All statistical analyses were performed using IBM SPSS Statistics for Windows, version 28.

3. Results & Discussion

A. Demographic characteristics

The mean age of patients recruited in this study was 64 years (41-82 years) and that of controls was 32 years (24-50 years). Majority of both patients and controls were males. Demographic and clincal data are shown in table 1. The tumors were mostly left sided (85.7%), moderately differentiated (71.4%) and were adenocarcinomas (91.4%). Most of the tumors were at stage III (51.4%), while 17.1% were advanced stage IV tumors. The mean CEA cencentration was observed to be high in majority of patients (≥5.0 ng/dL).

B. Serum cytokine concentrations

The mean concentration for IL-8 was 38.16 pg/ml (n=35) and it was higher than those of controls IL-8 = 33.67 pg/ml (n=35). The mean concentration for IL-6 was 46.31pg/ml (n=15) and for controls it was 11.15 pg/ml (n=15). There was no significant difference between the IL-8 levels of CRC patients and the control group ($p > 0.05$). Interestingly the mean serum concentration of IL-6 was found to be significantly higher in the CRC patients than controls ($p=0.04$). However, the sample number for IL-6 was 15 as there were descripancies between the duplicate values of some samples (CV>20). However, the research is ongoing to repeat the ELISA in those samples and to have more sample numbers (n=50). Similar observations have been reported in the previous studies (Shiga *et al*., 2016, Groblewska *et al*., 2008). The elevations of IL-6 have been hypothesized to be a causative factor of various cancers and to be related to prognosis (Giessen et al 2014 and Huang et al 2015).

C. Relationship of Serum cytokine concentrations with disease stage

Previous studies have reported a relationship between serum interleukin levels and disease status in CRC patients (Shiga *et al.,* 2016 and Yeh *et al.,* 2010). Chung et al. reported that tissue expression of IL-6 may represent a useful predictor of prognosis in CRC. In an attempt to identify a relationship of IL-8 and IL-6 with the

Table 1: Demographic and clinical characteristics of patients

Variables		Patients (P) (n=35)	Controls (C) (n=35)
	Age (Years)	41-82	24-50
	Mean	65 +/- 11	32 +/- 7
Gender	Male	24 (68.6%)	25 (71.5%)
	Female	11 (31.4%)	10 (28.5%)
Histology	Adenocarcinoma	32 (91.4%)	
	Singlet-ring cell carcinoma	1 (2.86%)	
	Not available	2 (5.71%)	
Grade	Well-differentiated	3 (8.6%)	
	Moderately differentiated	25 (71.4%)	
	Not available	7 (20%)	
Location	Right sided	3 (8.57%)	
	Left sided	30 (85.7%)	
	Transverse colon	2 (5.71%)	
Stage	I	1 (2.9%)	
	II	3 (8.5%)	
	III	18 (51.4%)	
	IV	6 (17.1%)	
	Not available 7	(20%)	

disease stage, it was observed that the mean serum [IL-6] in the CRC patients increase with the disease stage (Stage I: 0.16 pg/ ml; stage II: 7.01 pg/ ml; stage III: 15.8pg/ ml, and stage IV: 35.48pg/ ml). IL-8 did not show a positive relationship with the disease stage.

4. Conclusion

Although further studies are needed with higher sample numbers, data from this study provides clear preliminary evidence to use IL-6 as a potential biochemical marker for CRCs. Although previous studies have shown that IL-8 could be a potential marker for CRC, the data from the present study did not provide clear evidence to support previous findings for IL-8.

References

Akhmaltdinova, L., Sirota, V., Babenko, D., Zhumaliyeva, V., Kadyrova, I., Maratkyzy, M., Ibrayeva, A. and Avdienko, O., 2022. Proinflammatory cytokines and colorectal cancer – the impact of the stage.

Akira, S., Taga, T. and Kishimoto, T., 2022. Interleukin-6 in Biology and Medicine. Vainer N, Vainer, N., Dehlendorff, C. and Johansen, J., 2022. Systematic literature review of IL-6 as a biomarker or treatment target in patients with gastric, bile duct, pancreatic and colorectal cancer.

Burz, C., Bojan, A., Balacescu, L., Pop, V., Silaghi, C., Lupan, I., Aldea, C., Sur, D., Samasca, G., Cainap, C. and Chiorean, B., 2022. Interleukin 8 as predictive factor for response to chemotherapy in colorectal cancer patients.

Douaiher, J., Ravipati, A., Grams, B., Chowdhury, S., Alatise, O. and Are, C., 2022. Colorectal cancer-global burden, trends, and geographical variations.

Giessen, C., Nagel, D., Glas, M., Spelsberg, F., Lau-Werner, U., Modest, D., Michl, M., Heinemann, V., Stieber, P. and Schulz, C., 2022. Evaluation of preoperative serum markers for individual patient prognosis in stage I–III rectal cancer.

Gonzalez-Aparicio, M. and Alfaro, C., 2022. RETRACTED ARTICLE: Significance of the IL-8 pathway for immunotherapy.

Groblewska, M., Mroczko, B., Wereszczyńska-Siemiątkowska, U., Kędra, B., Łukaszewicz, M., Baniukiewicz, A. and Szmitkowski, M., 2022. Serum interleukin 6 (IL-6) and C-reactive protein (CRP) levels in colorectal adenoma and cancer patients.

Huang, Y., Liu, J. and Feng, J., 2022. The combination of preoperative serum C-reactive protein and carcinoembryonic antigen is a useful prognostic factor in patients with esophageal squamous cell carcinoma: a combined ROC analysis.

Iarc.who.int. 2022. Colorectal Cancer Awareness Month 2022 – IARC. [online] Available at: <https://www.iarc.who.int/news-events/colorectal-cancer-awareness-month-2022/> [Accessed 13 July 2022].

Jasperson, K., Tuohy, T., Neklason, D. and Burt, R., 2022. Hereditary and Familial Colon Cancer.

Klampfer, L., 2022. Cytokines, Inflammation and Colon Cancer.

Lichtenstein, P., Holm, N., Verkasalo, P., Iliadou, A., Kaprio, J., Koskenvuo, M., Pukkala, E., Skytthe, A. and Hemminki, K., 2022. Environmental and Heritable Factors in the Causation of Cancer — Analyses of Cohorts of Twins from Sweden, Denmark, and Finland.

Lin, Y., He, Z., Ye, J., Liu, Z., She, X., Gao, X. and Liang, R., 2022. <p>Progress in Understanding the IL-6/STAT3 Pathway in Colorectal Cancer</p>.

Long, A., Lundsmith, E. and Hamilton, K., 2022. Inflammation and Colorectal Cancer.

Nagasaki, T., Hara, M., Nakanishi, H., Takahashi, H., Sato, M. and Takeyama, H., 2022. Interleukin-6 released by colon cancer-associated fibroblasts is critical for tumour angiogenesis: anti-interleukin-6 receptor antibody suppressed angiogenesis and inhibited tumour–stroma interaction.

Park, J., Chang, H., Yeo, H., Han, N., Kim, B., Kong, S., Kim, J. and Oh, J., 2022. *The relationships between systemic cytokine profiles and inflammatory markers in colorectal cancer and the prognostic significance of these parameters*.

Pretzsch, E., Bösch, F., Neumann, J., Ganschow, P., Bazhin, A., Guba, M., Werner, J. and Angele, M., 2022. Mechanisms of Metastasis in Colorectal Cancer and Metastatic Organotropism: Hematogenous versus Peritoneal Spread

Rattray, N., Charkoftaki, G., Rattray, Z., Hansen, J., Vasiliou, V. and Johnson, C., 2022. Environmental Influences in the Etiology of Colorectal Cancer: the Premise of Metabolomics

Rubie, C., 2022. Correlation of IL-8 with induction, progression and metastatic potential of colorectal cancer.

Shrihari, T., 2022. Dual role of inflammatory mediators in cancer.

Shiga, K., Hara, M., Nagasaki, T., Sato, T., Takahashi, H., Sato, M. and Takeyama, H., 2022. Preoperative Serum Interleukin-6 Is a Potential Prognostic Factor for Colorectal Cancer, including Stage II Patients.2003.

Shiga, K., Hara, M., Nagasaki, T., Sato, T., Takahashi, H., Sato, M. and Takeyama, H., 2022. Preoperative Serum Interleukin-6 Is a Potential Prognostic Factor for Colorectal Cancer, including Stage II Patients.

Sun, Q., Shang, Y., Sun, F., Dong, X., Niu, J. and Li, F., 2022. Interleukin-6 Promotes Epithelial-Mesenchymal Transition and Cell Invasion through Integrin β6 Upregulation in Colorectal Cancer.

Sung, H., Ferlay, J., Siegel, R., Laversanne, M., Soerjomataram, I., Jemal, A. and Bray, F., 2022. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries.

Tseng-Rogenski, S., Hamaya, Y., Choi, D. and Carethers, J., 2022. Interleukin 6 Alters Localization of hMSH3, Leading to DNA Mismatch Repair Defects in Colorectal Cancer Cells.

T, K., H, I. and C, M., 2022. Serum interleukin-6 level reflects the tumor proliferative activity in patients with colorectal carcinoma. [online] PubMed. Available at: <https://pubmed.ncbi.nlm.nih.gov/10375098/> [Accessed 13 July 2022].

Waldner, M., Foersch, S. and Neurath, M., 2022. Interleukin-6 - A Key Regulator of Colorectal Cancer Development.

Wang, Y., Ding, Y., Deng, Y., Zheng, Y. and Wang, S., 2022. Role of myeloid-derived suppressor cells in the promotion and immunotherapy of colitis-associated cancer.

Xia, W., Chen, W., Zhang, Z., Wu, D., Wu, P., Chen, Z., Li, C. and Huang, J., 2022. Prognostic Value, Clinicopathologic Features and Diagnostic Accuracy of Interleukin-8 in Colorectal Cancer: A Meta-Analysis.

Yamagishi, H., Kuroda, H., Imai, Y. and Hiraishi, H., 2022. Molecular pathogenesis of sporadic colorectal cancers.

YC, C., YL, C. and CP, H., 2022. Clinical significance of tissue expression of interleukin-6 in colorectal carcinoma. [online] PubMed. Available at: <https://pubmed.ncbi.nlm.nih.gov/17094421/> [Accessed 13 July 2022].

Yeh, K., Li, Y., Hsieh, L., Lu, C., Chou, W., Liaw, C., Tang, R. and Liao, S., 2022. Analysis of the Effect of Serum Interleukin-6 (IL-6) and Soluble IL-6 Receptor Levels on Survival of Patients with Colorectal Cancer.

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