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Biochemical Study of IL 12, IL 1B and IL 6 cytokines as Diagnostic Factors for Bladder Cancer in Egyptian Patients

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Abstract: The cancer of the bladder (UBC) is the most prevalent site of cancer in the urinary system. UBC is roughly three times more prevalent in males than in women, with the majority occurring in industrialized nations, but growing in emerging countries due to lifestyle changes. Few cytokines have an important role in cancer formation. **Aim:** to evaluate the role of interleukin 6, interleukin 12 and interleukin 1B as diagnostic and Prognostic Factors for UBC in Egyptian Patients**. Methods:** A case-control study included 100 patients with UBC and 100 healthy control participants. We used ELISA to measure the levels of IL 6, IL 12, and IL 1B in those patients and controls. **Results:** The UBC group had considerably higher median levels of IL-12, IL-1B, and IL 6 compared to the control group (p-value < 0.001), demonstrating a statistically significant relationship between these cytokines and UBC. **Conclusion:** High level of IL-6, IL-12 and IL1B were associated with an increased risk of UBC.

keywords: bladder cancer, interleukin, cytokines, IL 6, IL1B, IL 12.

1.Introduction

Urinary bladder cancer (UBC) is one of the most prevalent tumours within the lower urinary tract; it is the ninth most often diagnosed malignancy in the globe, with the greatest rates in Southern and Eastern Europe, Africa, the Middle East, and North America. The most frequent urinary tract malignancy is the UBC. It is one of the most prevalent cancer in men and women (1). In Egypt, UBC has been the most frequent malignancy over the last 50 years. In 2021, the United States is expected to have 83,730 new cases of UBC and 17,200 fatalities (2). In Egypt, UBC is the third most frequent cancer, second among men and seventh among females, with age-standardized rates of 21.1 and 5.5 per 100,000/year in either sex (3).

Hematuria is a common sign of UBC that typically starts unexpectedly and is painless. It can be further classified as asymptomatic or symptomatic (4). Irritative symptoms include dysuria, urgency, urge incontinence, and frequency, as well as obstructive signs (5). UBC is classified into three types based on muscular layer invasion: (i) superficial or non-muscle-invasive UBC (NMIBC); (ii) muscle-invasive bladder cancer (MIBC); and (iii) metastatic disease (6). The American Joint Committee on Cancer (AJCC) Staging Manual 8th edition (7) categorizes patients with UBC depending on tumour grade and stage. The TNM classification is now the standard staging method for UBC, and it is based on clinicopathological information. The degree of invasion as well as the number and location of metastatic lymph nodes determine whether UBC is categorized as T or N. The occurrence of distant metastases defines the M category (8).

The most common histological kind of UBC is pure urothelial carcinoma, which can be classed as low or high grade. The latter are divided into those with and without NMIBC and MIBC (9). The tumour, node, and metastasis (TNM) staging system divides NMIBC into three types: The papillary form

(Ta), the carcinoma in situ (CIS) and T1 (which invades the sub-epithelial connective tissue) (10).

UBC has a complicated etiopathogenesis that is influenced by a variety of variables, including chemical carcinogens in the diet, past therapies, and genetic and inherited factors (11). Interleukin-6 (IL-6) is a 184-amino acid multifunctional proinflammatory cytokine encoded by the human IL-6 gene on chromosome 7p15–p21. IL6 levels have been found to be raised in advanced stage cancer, and these levels have previously been related to an elevated risk of cancer (12). IL-12 is largely generated by activated inflammatory cells. Numerous molecular epidemiology investigations have investigated the potential associations between IL-12 polymorphisms and different malignancies (13).

IL-1β, a pro-inflammatory cytokine, plays a significant role in carcinogenesis by modulating host-environment interactions. IL-1β has several impacts on cancer, including immune cells, angiogenesis, cell proliferation, migration, and metastasis (14). Cystoscopy is the most frequent urological technique, allowing visualization of the urethra and bladder walls in order to assess urine complaints (15). Transurethral bladder tumour (TURBT) is the standard technique for diagnosing and treating bladder tumours (16). In terms of treatment, the most common treatments are surgery, intravenous chemotherapy, systemic chemotherapy, reconstruction, radiation therapy, immunotherapy, and combination therapies (17). Most bladder malignancies are initially treated with transurethral resection (TUR) or tumour removal. Repeat transurethral resection (reTUR) is advised for high-risk NMIBC to eliminate remaining disease and enhance cancer outcomes. The standard treatment for NMIBC is TURBT with intravesical adjuvant chemotherapy or immunotherapy (18). Radical cystectomy has long been regarded the "gold standard" in the treatment of localized MIBC (19). While, the 5-year survival rate is just around 50%. Additional medications, such as chemotherapy or immunotherapy, are required to reduce the risk of recurrence. Currently, immunotherapy or intravesical chemotherapy is the standard of care with intermediate- and high-risk NMIBC; they are typically delivered via a bladder catheter, which is known as intravesical therapy (20).

2. Materials and methods

Study participants:

A case-control research was undertaken on 100 UC patients ranging in age from 39 to 64 years old, with a mean of 49 years, and 100 healthy matched controls. Their average age was 43 years (35-57). Patients were selected from the Outpatients' Clinic of Urology and Nephrology Centre at Mansoura University in Egypt. The local ethical and scientific committees approved the study, and all participants provided informed permission. The patient's information included age, gender, domicile, parental consanguinity, family history of breast cancer, occupation, education, and laboratory tests.

Sampling:

All patients and controls received five milliliters of venous blood by a clean venipuncture with plastic disposable syringes. Each blood sample was separated into three tubes, with 2 mL transferred to a simple free plastic collection tube. Blood was allowed to clot before being centrifuged at 3000 rpm for 10 minutes. Serum was then extracted for clinical chemistry testing.

Methods:

Semi-automated devices from BIOTEK, USA were used to quantify interleukins (IL-iβ, IL-6, and IL-12). As a self-contained and programmed device, the ELx50 Washer provides complete control over exact fluid distribution, from the delicate trickling of a simple squeeze bottle to the full power of pressure delivery systems. The ELx50 is a versatile and modular design that has unparalleled 96- and 384-well strip or plate cleaning capabilities. The ELx800 Automated Microplate Reader is a single-channel reader-assay device that automates endpoint analysis for a wide range of ELISA-based applications. INNOVA Biotech (Beijing, China) acquired the used ELISA Kits for Interleukins IL-iβ, IL-6, and IL-12, and followed the manufacturer's instructions.

Statistical analysis

The data were analyzed using "IBM SPSS Statistics for Windows, Version 25.0, Armonk, NY: IBM Corp." To evaluate the significance of the difference between the two study groups, parametric and nonparametric variables were tested using the T Student and Mann Whitney tests, respectively. To observe the relationship between qualitative variables, the Chi-Square test was used. The ROC Curve (receiver operating characteristic) was designed to evaluate the validity of quantitative variables. A p-value less than 0.05 is considered significant.

3. Results and Discussion

RESULTS

The present study comprised 100 UBC patients and 100 healthy controls of similar age and gender. There were no significant variations in hematologic and biochemical markers between the two groups, except BC patients who appeared to have microcytic hypochromic anemia, which might be an anemia of chronic disease **(Table 1).**

The mean±SD of IL-12 for the UBC group is 28.45±17.24, while it is substantially higher at 93.09±39.86 in the group without UBC. The median IL-12 level for the UBC group is 21.31, ranging from 3.48 to 69.57, whereas the median for the non-cancer group is notably higher at 102.61, with a range of 2.61 to 153.91. The significant difference indicates a statistically significant association between IL-12 levels and UBC **(Figure 1A).**

The mean±SD of IL-1β is 9.59±2.94 in the UBC group, while it rises to 18.3±9.95 in the non-cancer group. The median for the UBC group is 8.9, ranging from 5.3 to 16.6, whereas the non-cancer group has a median of 14.35, with a range of 6.5 to 50.9, with a significant difference indicating an association between IL-1B levels and UBC **(Figure 1B).**

Regarding IL-6 levels, the mean±SD is 0.98±0.44 in the UBC group, while it substantially increases to 4.61±2.7 in the non-cancer group. The median IL-6 level for the UBC group is 0.89, ranging from 0.05 to 2.14, whereas the median for the non-cancer group is 4.2, with a range of 0.8 to 10.8. The significant difference indicated a statistically significant association between IL-6 levels and UBC **(Figure 1C).**

Overall, the data presented in the table demonstrate a clear association between elevated levels of IL-12, IL1B, and IL-6 and UBC. These findings suggest that these interleukins could potentially serve as biomarkers for the disease.

Significant positive correlations were observed between IL-12, IL1β and IL-6 level among BC group ($p<0.001$ for each 2 pairs). While, no significant correlations were found between IL-12, IL1β and IL-6 level with each other among control group ($p>0.05$ for each two pairs) **(Figure 2).**

Receiver operating characteristic (ROC) curve of IL-12, IL1B and IL-6 levels was conducted for discrimination between BC cases and control groups. For IL-12, the AUC is 0.894 with a 95% CI of 0.843 - 0.933, indicating moderate diagnostic ability. The p-value is less than 0.001, suggesting a significant difference between UBC cases and controls. The sensitivity, specificity, PPV, NPV, and accuracy are also provided, showing that IL-12 has high values across these metrics. IL1B has an AUC of 0.821 with a 95% CI of 0.761 - 0.872 and a p-value less than 0.001, indicating moderate diagnostic ability. The sensitivity, specificity, PPV, NPV, and accuracy are lower compared to IL-12 but still demonstrate some ability to differentiate between UBC cases and controls. IL-6 shows the highest AUC of 0.951 with a 95% CI of 0.911 - 0.976 and a p-value less than 0.001, indicating high diagnostic ability.

The sensitivity, specificity, PPV, NPV, and accuracy are also high for IL-6, suggesting it is a strong discriminator between UBC cases and controls. The comparisons between AUCs for IL-12, IL1B and IL6 are also provided. These comparisons show that IL-6 has significantly higher discriminatory ability than IL1B and IL-12 ($p=0.040$, <0.001 respectively) in distinguishing between UBC cases and controls. In addition, IL-12 had significantly higher discriminatory ability than IL 1B ($p=0.022$).

In summary, the table demonstrates that IL-12, IL1B, and IL-6 levels have varying degrees of validity for discriminating between UBC

cases and control groups, with IL-6 showing the highest discriminatory ability among the three interleukins **(table 2 and figure 3).**

Discussion

Urinary bladder cancer (UBC) is the most prevalent tumour in the urinary tract. The World Health Organization (2016) divides bladder tumours into two categories: low grade and high grade tumors. The differentiation between low and high-grade urothelial disease has consequences for patient risk classification and therapy (21). UBC has a complicated etiopathogenesis, including chemical carcinogens, nutrition, prior therapies, and genetic and inherited variables (11). The current study aims to assess the function of interleukin 6, interleukin 12, and interleukin 1B as diagnostic and prognostic factors for UBC in Egyptian patients. The current investigation found that the UBC group had considerably greater IL-12 levels than the control group. These data show that IL12 could be used as a disease biomarker. In accordance with Kovacs, who discovered that IL-12 was highly raised in all tumour stages, both before and after further chemo/radiotherapy. They showed a good correlation with the advancement. This study demonstrates for the first time that IL-12 is implicated in cancer illnesses (22). IL-12 is an immunomodulatory cytokine that is essential for the host's immune response to cancer (23). Despite its anticancer properties, IL-12 can stimulate tumour development by directly up-regulating proangiogenic proteins, which aid in tumour cell epithelial-mesenchymal transition and migration. However, there was an elevated risk of developing cancer in psoriasis patients treated with high doses of anti-IL-12/23p40 mAbs (24). The current investigation found that the UBC group had considerably greater IL1B levels than the control group. These findings imply that these interleukins could be used as disease biomarkers. In accordance with Kuo and colleagues, who discovered that patients with UBC and control individuals generated IL-1B in a dose-dependent manner, patients with UBC had increased IL-1B activities, although statistical significance was not attained when compared to controls. This investigation found that individuals with UBC do not spontaneously produce IL-1B (25). Instilling LPS/PS repeatedly can cause dysfunction of voiding, denudation of urothelium, and detrusor muscle fibrosis by upregulating the IL-1β pathway and increasing the transition process of epithelial-mesenchymal in bladder tissues. Downregulating the IL-1β-related pathway in bladder tissues can significantly reduce bladder damage (26). Interestingly, it was shown that the proinflammatory cytokines IL1A and IL1B are most likely linked to UBC invasion (27). The current investigation found that the UBC group had considerably greater IL-6 levels than the control group. These findings imply that these interleukins could be used as disease biomarkers. In line with Al-Humairi et al.'s discovery that median levels of IL-6 were significantly higher in newly diagnosed UBC cases than in healthy controls, IL-6 levels were found to be higher in patients over the age of 50, females, cigarette smokers, and patients with no family history of cancer. The ROC curve analysis showed that IL-6 is a highly accurate predictor of UBC. In this case, IL-6 exhibited diagnostic sensitivity and specificity of around 80.0%. (28). Wei et al. discovered that the IL6 level is higher in the sera of patients with several types of malignancies, including UBC, and is thought to be related with poor clinical outcomes. They proposed that cells were the source of IL-6 secretion and that IL6 may regulate cells' stem-like properties (29). In a mouse model of UBC, recombinant IL-6 inhibited tumour development in a dose-dependent way. Furthermore, xenograft animal experiments demonstrated that IL-6 overexpression was related with decreased carcinogenesis in bladder cells, and IL-6 knockdown reversed this effect (30). The study found that there were significant positive correlations between IL-12, IL1β, and IL-6 levels among the BC group, but not in the control group. This could be due to the disturbed microenvironment and immune dysregulation in cases of BC, indicating an active immune response and inflammation within the tumour. These cytokines may have key functions in tumour development, invasion, and immune evasion. IL-12 has an important function in immune response control. IL-12, IL1B, and IL-6 levels in the current investigation had no significant relationships with demographic or laboratory characteristics. The current investigation found that IL-6 and

IL-12 have modest diagnostic ability. While IL6 levels have a strong diagnostic capability. IL-12, IL1B, and IL-6 levels exhibit variable degrees of validity for distinguishing UBC patients from control groups, with IL-6 having the most discriminatory capacity of the three interleukins. The disparities in outcomes between research could be related to various ethnic origins.

The current study had certain limitations, including a small sample size and the fact that it was the first to investigate the effect of all of these cytokines in the same patients with UBC. Multiple case control studies should be undertaken to corroborate the current findings. In conclusion, the current investigation found that blood levels of IL-12, IL1B, and IL-6 were linked to an elevated risk of UBC and served as biomarkers for disease susceptibility.

Table 1: Comparison between patients and control group regarding personal history.

	Control n = 100		**Bladder Cancer n = 100**		***p***
	Mean ± SD	**Median (min-max)**	**Mean ± SD**	**Median (min-max)**	
Male	90(90%)		84(84%)		0.207
Female	10(10%)		16(16%)		
Age (years)	52.73 ± 8.54	54.0 (33.0 – 68.0)	53.56 ± 8.35	55.0 (33.0 – 68.0)	0.488
WBC ($X10^9$/L)	7.67 ± 1.90	7.75(4.20 – 10.70)	8.33 ± 3.06	7.5(3.60 – 22.70)	0.430
RBC ($X10^{12}$/L)	4.49 ± 0.36	4.45(3.60 – 5.50)	3.82 ± 0.52	3.84(2.40 – 5.12)	<0.001*
Hemoglobin (g/dL)	13.59 ± 1.35	13.55(11.30 – 15.90)	10.61 ± 1.96	10.68(4.24 – 13.84)	<0.001*
Hematocrit (%)	44.02 ± 2.54	44.2(39.60 – 48.0)	39.30 ± 5.97	40.3(18.60 – 51.30)	<0.001*
MCV (fL)	88.55 ± 3.64	88.35(82.30 – 95.60)	82.90 ± 7.51	83.7(53.0 – 100.8)	<0.001*
MCHC (g/dL)	34.21 ± 1.11	34.35(32.10 – 35.90)	28.0 ± 3.47	28.7(17.50 – 35.0)	<0.001*
Platelets ($X10^9$/L)	274.8 ± 85.43	269.5(142.0 – 450.0)	216.0 ± 114.7	196.5(27.30 – 534.0)	<0.001*
Creatinine (mg/dL)	1.01 ± 0.23	1.1(0.60 – 1.30)	1.09 ± 0.55	1(0.40 – 3.70)	0.575
ALT (U/L)	17.25 ± 6.33	17(2.6 – 28.0)	21.94 ± 14.82	17(2.60 – 88.0)	0.219
AST (U/L)	18.47 ± 5.13	19(7.0 – 27.0)	24.57 ± 20.42	19(7.0 – 167.0)	0.115
ALP (U/L)	79.46 ± 24.47	76(38.0 – 149.0)	79.53 ± 24.68	76(38.0 – 156.0)	0.998
Albumin (g/dL)	4.25 ± 0.41	4.2(3.60 – 4.90)	4.05 ± 0.45	4.1(2.90 – 5.0)	0.101
RBG (mg/dL)	106.8 ± 14.27	107.6(81.34 – 128.4)	141.7 ± 79.93	107.5(77.0 – 460.0)	0.431
Uric acid (mg/dL)	5.08 ± 1.26	5.1(2.65 – 7.06)	5.81 ± 1.39	5.6(3.0 – 9.20)	0.521
K (mmol/L)	4.58 ± 0.31	4.7(4.10 – 5.10)	4.19 ± 0.54	4.2(2.90 – 5.90)	0.367
Na (mmol/L)	140.1 ± 2.04	140(136.0 – 144.0)	137.2 ± 4.0	138(120.0 – 145.0)	0.198

SD.: Standard deviation, Min.: Minimum, Max.: Maximum.

Table 21.Validity of IL-12, IL1B and IL-6 levels for discrimination between BC cases and control groups.

	IL-12	**IL-1β**	**IL-6**
AUC	0.894	0.821	0.951
95% CI	0.843 - 0.933	0.761 - 0.872	0.911 - 0.976
P	<0.001	<0.001	<0.001
Cut off	>53.91	>11.6	>1.28
Sensitivity (%)	85	72	90
Specificity (%)	90	76	86
PPV (%)	89.5	75.0	86.5
NPV (%)	85.7	73.1	89.6
Accuracy (%)	87.5	74.0	88.0
P2	-	0.022	0.040
P3	-	-	<0.001

AUC, area under ROC curve; CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value; p1, probability of discrimination between cases and controls; p2, comparison between AUCs versus IL-12 AUC; p3, comparison between AUCs versus IL-1B AUC.

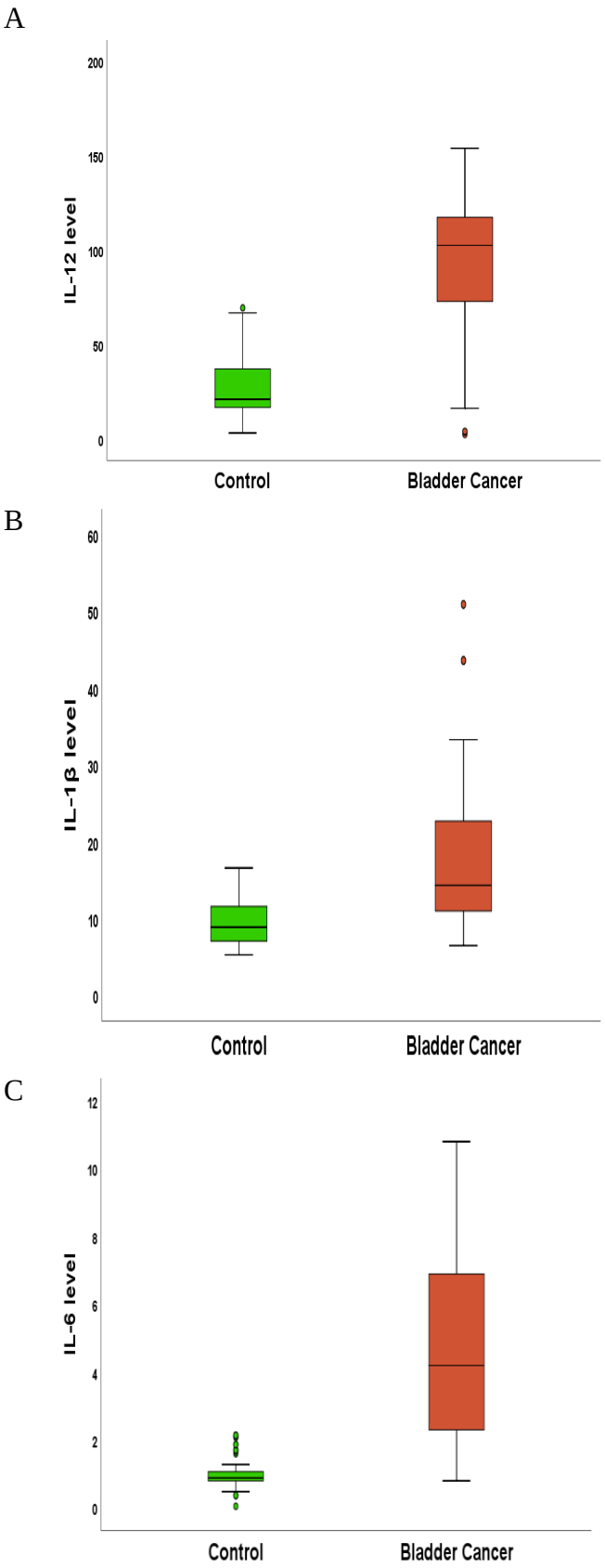

Figure 1: Boxplot for comparison between patients and control group regarding (A) IL-12, (B) IL-1β, and (C) IL-6 levels.

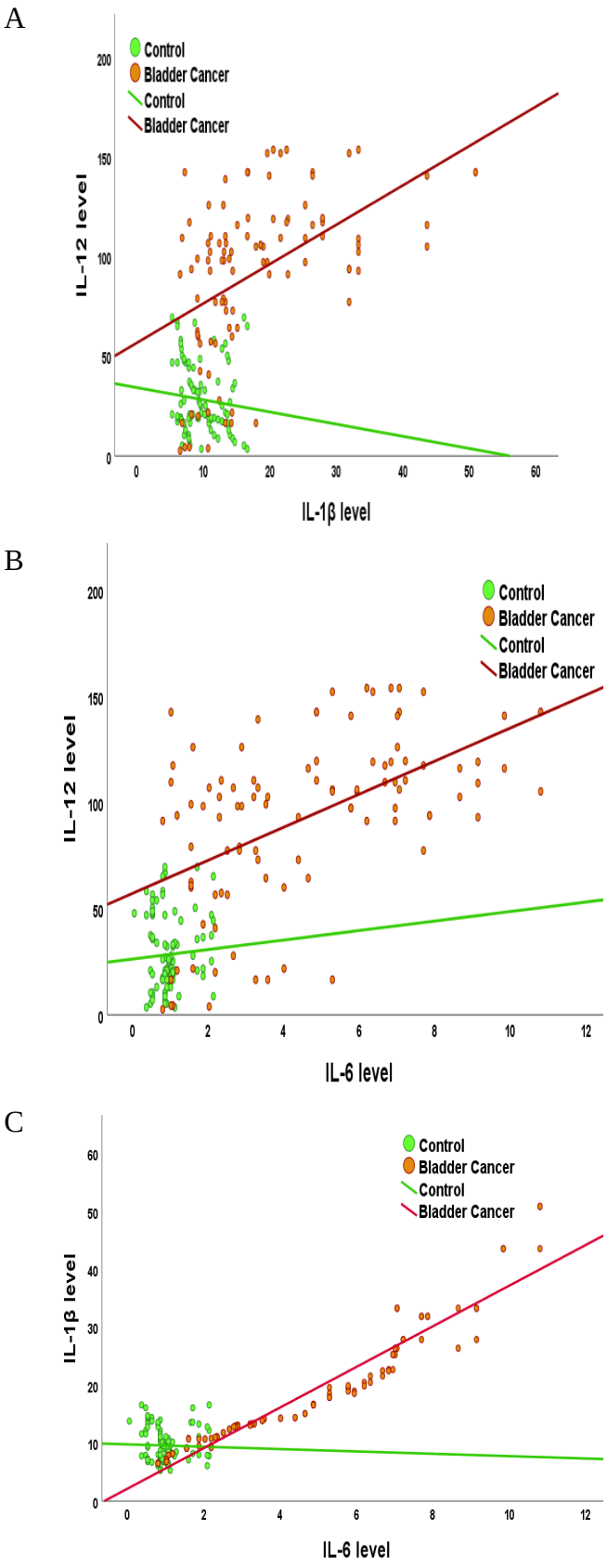

Figure 2: Scatter plot for correlation between (A) IL-12 and IL-1B, (B) IL-12 and IL-6 and (C) IL-1β and IL-6 among patients and control groups.

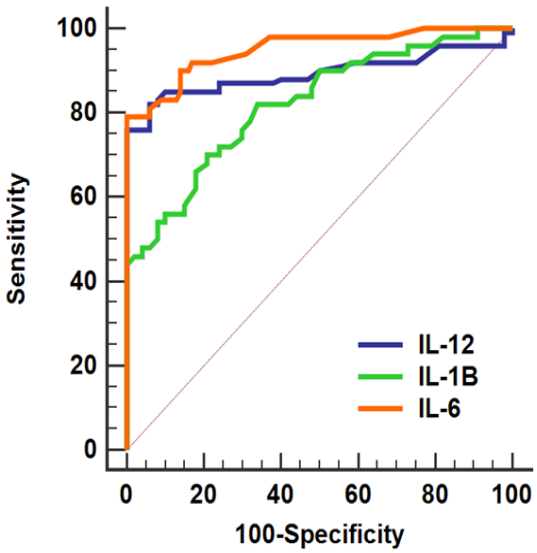

Figure3: ROC of IL-12, IL1B and IL-6 levels for discrimination between BC cases and control groups.

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