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Basic Research

Role of Urinary Apolipoprotein A1 level in Predicting Development of Cancer of Urinary Bladder

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Abstract:

Background: *Urinary bladder cancer is the tenth most common malignant condition globally. Now incidence and prevalence of bladder cancer is gradually increasing. Urinary biomarker can play an important role for the diagnosis of urinary bladder cancer. Urinary apolipoprotein A-1 is a non–invasive biomarker. In this study, we evaluate the role of urinary apolipoprotein A-1 in predicting development of urinary bladder cancer.*

Methods: *This cross-sectional comparative study was conducted in the Department of Laboratory Medicine, department of Urology, BMU. Total study subjects were 52 in number of which 26 were urinary bladder cancer patients (group I) and 26 were healthy subjects (group II). After taking informed written consent, 5 ml of urine was collected for estimation of urinary apolipoprotein A-1 in the Department of Laboratory Medicine by ELISA principle. After data collection and processing, all statistical analysis were done by SPSS version 22.0.*

Results: *The mean urinary apolipoprotein A-1 level was found 47.95±41.90 ng/ml in group I and 6.21±7.01 ng/ml in group II. The difference was statistically significant ($p< 0.05$) between two groups as evidenced by the unpaired t-test. In ROC curve analysis a cut off value of 20.28 ng/ml for urinary apolipoprotein A-1 showed highest sensitivity (70.83%) and specificity (92.85%). The area under curve (AUC) of urinary apolipoprotein A-1 level was 0.899, indicating its effectiveness in predicting development of malignancy of urinary bladder.*

Conclusion: *Urinary apolipoprotein A-1 level can be a promising biomarker in predicting development of urinary bladder.*

Key words: *Urinary Bladder Cancer (UBC), Urinary Apolipoprotein A-1 (Apo A1).*

Background:

Urinary bladder cancer (UBC) is the tenth most common malignant condition globally[1]. It is the second most urothelial cancer with high recurrence and poor prognosis rate[2]. Now a days, incidence and prevalence of bladder cancer gradually increasing[3]. In western world 4.7% of urinary bladder cancer contributing among the all cases of cancer as well as morbidity and mortality. The prevalence of bladder cancer in Egypt 16% in male, producing 7900 deaths annually. In Asia, total cases of incidence 696239 annually, among them 68.1% male and 31.9 % female. In India, the incidence of bladder cancer is 2.25% / 100000 annually[4]. In Bangladesh, 991 bladder cancer death was recorded by the year of 2020[5].

Histologically, primary bladder cancer was classified into urothelial cancer, squamous cell carcinoma and adenocarcinoma[6]. Among about 90% are urothelial cancer[7]. The main etiological factor for urinary bladder cancer is cigarette smoking[8]. Occupational exposure to urothelial carcinogen like benzidine, toluidine 2, naphthylamine, aminobiphenyl etc. having remarkable risk of bladder cancer. Occupation associated risk factor of bladder cancer are textile worker, dye worker, petrol workers, painters, chemical workers and sewage worker.

Bladder cancer has a complex multifactorial pathophysiology. Chronic inflammation like cystitis causes initiation and progression of underlying pathophysiology of invasive and metastatic cancer[9]. Schistosoma haematobium, a blood flukes having remarkable risk factor for urinary bladder cancer. Genetic and epigenetic alteration or its cellular pathway signaling contributing pathophysiology of progression or recurrence of bladder cancer[10]. Series of genetic events play an important role in bladder cancer like activation of oncogene ras, c-erb-1, 2 and E2F3, inactivation of tumor suppressor gene p53, p21, p16 and RB gene having potential risk of bladder cancer[11].

Current gold standard diagnostic tool for urinary bladder cancer is cystoscopy guided biopsy but it is invasive, painful, time consuming and costly procedure[12]. In addition, this procedure may cause urinary tract infection, mucosal irregularity of urinary bladder and small area of carcinoma in situ may be escaped, significant false negative result also may occur due to operating error[13]. Current non-invasive diagnostic tool for UBC is urine for cytology but it is less sensitive (37%) and highly specific (90%). Sensitivity and specificity is also low for low grade tumor[14]. Other diagnostic tools for diagnosis of bladder cancer is bladder cell antigen and Nuclear Matrix Metalloproteinase 22, both are showing sensitivity and specificity are 50% ,70% and 80%, 75% respectively[15]. So more reliable and less invasive diagnostic methods should be found out for the diagnosis of urinary bladder cancer[16].

Apolipoprotein A1 (Apo A-1) is one of the urinary

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non-invasive biomarkers. Apo A-1 is synthesized by liver and intestine[17]. It is the major part of structure of high-density lipoprotein. It plays an important role in transport of cholesterol from periphery to liver. It has also an important role in immunity, inflammation, apoptosis and anti- tumor effect[10]. Apo A1 comes from Lysophospholipid which is the part of HDL that may be connected with phospholipid having basic role in causation of malignancy and accounted as biomarker of cancer[18].One study showed that sensitivity and specificity of Apo A-1 in UBC was 90.7% and 90% respectively[7]. Another study showed sensitivity and specificity was 83.7% and 89.7% respectively[19].

Urinary biomarkers play an important role in detection of urinary bladder cancer. Moreover, sample collection of urine is an outpatient procedure, easy and non-invasive technique. It also gives quick result detection for urinary bladder cancer[11]. Apo A-1 is also measured to diagnose non -malignant condition of UB but serum Apo A-1 level cannot differentiate from malignant and nonmalignant conditions of urinary bladder[20]. The aim of this study is to evaluate urinary apolipoprotein A1 as a biomarker in predicting development of urinary bladder cancer.

Methods:

This cross-sectional comparative study was conducted in the department of Laboratory Medicine and department of Urology of BMU for a period of one year (March 2023 to February 2024).

Total study subjects were 52 in number of which 26 were urinary bladder cancer patients (group I) and 26 were healthy subjects (group II). Those who were diagnosed case of urinary bladder cancer confirmed by cystoscopy guided biopsy with an age of more than or equal to 20 years of either sex were included in group I. Healthy Subjects with an age of more than or equal to 20 years of either sex were included in group II. Patients having any history of other malignancy or having history of radiological intervention due to any other cause were excluded from the study.

After ethical clearance from Institutional Review Board (IRB), study population were selected from inpatient and outpatient department of Urology, BMU. Diagnosed cases of urinary bladder cancer and healthy subjects were included in this study who met the inclusion and exclusion criteria. Full explanation regarding the aims and objective of this study and necessity of investigation was informed to each participant of the study. Informed written consent were taken from each study subjects. The brief history taking, physical and clinical examination were done. Random urine sample was collected and labeled with identification number. After centrifuged of urine 3000 RPM for 5 min, supernatant was kept for urinary Apo A1 estimation. Urinary Apo A1 was measured by semiautomated ELISA reader by the principle of Sandwiched enzyme linked immunosorbent assay in the Department of Laboratory Medicine, BMU. Comparison of urinary Apo A-1 was done between diagnosed cases of bladder cancer and healthy subjects.

Urinary Apo A1 was measured by semiautomated ELISA reader by Sandwiched ELISA principle on three successive days (17 Samples in each day and t3 was control) and then comparison was done in two groups. Data was collected using a pre-designed data collection sheet for statistical analysis and interpretation. Data and results were presented in the form of tables, figures and diagrams where applicable.

Statistics

Statistical analyses were carried out by using the Statistical Package for Social Sciences version 22.0 for Windows (SPSS Inc., Chicago, Illinois, USA). Variables were expressed as frequencies and percentages. Chi-square (X^2) test and unpaired t test was done to evaluate the association between variables. ROC curve was plotted for evaluating the effectiveness of urinary apolipoprotein A-1 in predicting development of urinary bladder. A p-value <0.05 was considered as significant.

Results:

The main objective of the study was to evaluate urinary apolipoprotein A-1 in predicting development of urinary bladder. Total 52 study subjects were enrolled in this study. Among them 26 were cancer of urinary bladder and 26 were healthy subjects which were placed in group I and group II respectively. Data was collected through a pre-designed data collection sheet. Data were gathered, compiled, edited and plotted in table and figure. The mean (±SD) age was 56.92±14.65 and 38.81±10.47 in Group I and Group II respectively. In group I 25 (96.2%) were male and 1 (3.8%) was female, and in group II, 21 (80.8%) were male and 5 (19.2%) were female.

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Table I: Comparison of apolipoprotein-A1 in study subjects (N=52)

Apolipoprotein	Study subjects (N=52)		p value
-A1 (ng/ml)	Group I (n=26)	Group II (n=26)	
Mean ±SD	47.95±41.90	6.21±7.01	0.001

Unpaired t-test was done to measure the level of significance.

Table I shows that the mean (± SD) of urinary apolipoprotein A-1 level was 47.95±41.90 ng/ml in group I. In group II, mean (± SD) of urinary apolipoprotein A-1 was 6.21±7.01ng/ml. The difference of mean between two group was statistically significant (p=0.001), as shown by unpaired t test.

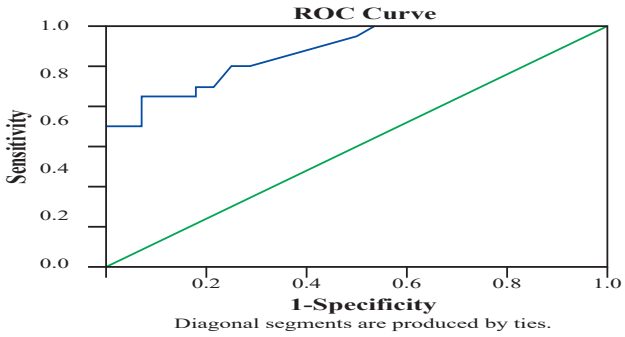

Figure 1: ROC curve showing effectiveness of urinary apolipoprotein A-1 in predicting development of urinary bladder carcinoma

ROC curve analysis of urinary apolipoprotein A-1 (fig 1) showed an AUC value of 0.899 (95% CI: 0.819-0.979) which was statistically significant (P <0.05).

Table II: Evaluation of different cut off points of urinary apolipoprotein A-1 as a predictor of development of UBC

Cutoff value	Sensitivity	Specificity	PPV	NPV	Accuracy	Youden index (j=sen+spe-1)
18.72	0.708	0.821	78.15%	72.47%	70.08%	0.530
20.28	0.708	0.93	92%	89.47%	82.69%	0.637
23.30	0.583	0.071	69.33%	72.14%	67.11%	0.512

Table II shows the cut-off value of ≥20.28 showed the highest Youden index (0.637) with sensitivity 71%, specificity 93%, PPV 92%, NPV 89.47% and accuracy of 82.69%.

Table III: Performance test of urinary Apolipoprotein A-1 in predicting UBC

Apo A1	Study subjects (N=52)		Total	P value
	Group I (n=26)	Group II (n=26)		
≥20.28	17 (89.5%)	2 (10.5%)	**19**	
<20.28	9 (21.2%)	24 (78.8%)	**33**	**0.001**
Total	**26**	**26**	**52**	

Data expressed as frequency (percentage); Chi-square test was done to measure the level of significance.

Discussion:

Urinary bladder cancer is the second most common urological malignancy and 6th most common cancer in male population worldwide. Bladder cancer affected more than 5,40,000 people worldwide and has become a growing healthcare problem.[4] Urothelial cancer has strong association with environmental risk factors with age and male sex pre-dominance. The ratio of male to female is 4:1. Incidence and prevalence of bladder cancer rises with advancing age and most common in six to eight decades of life.[22]

In western world, urinary bladder cancer is contributing about 4.7% among the all cases of cancer as well as morbidity and mortality. The prevalence of bladder cancer in Egypt 16% in male, producing 7900 deaths annually. In Asia, total cases of incidence 696239 annually, among them 68.1% are male and 31.9 % are female. In India, the incidence of bladder cancer in India is 2.25% / 100000 annually.[4] In Bangladesh, 991 cases bladder cancer death is recorded by the year of 2020.[5]

Cystoscopy is a procedure that allows urologist to examine urinary bladder and the tube that carries urine out of body through urethra. Cystoscopy is one of the invasive procedures used to detect UBC. Among the noninvasive tests, Urine cytology is a test to screen urine for cancer cells. This is one of tools used to diagnose cancers in the urinary tract, including bladder, kidney, prostate, ureter and urethra cancers. A urine cytology test alone can't diagnose cancer. The sensitivity of voided urine cytology for detection of urothelial neoplasm ranges from 18% to 76% and is dependent on factors such as tumor grade, the number of specimens examined, and the expertise of the cytopathologist. Specificity of urine cytology is as high as 93%.

Another noninvasive test to detect UBC is detection of bladder cell antigen in urine, which is used for evaluating the patient with urinary bladder cancer and recurrent bladder cancer. The presence or absence of BCA is not an absolute indicator of bladder cancer. It is also increased in trauma to bladder, UTI, and bladder calculi can result in false positive results.

Measuring urinary Nuclear Matrix protein 22 (NMP22) is another noninvasive way of diagnosing UBC. It is one of the nuclear matrix proteins, which is specific in urothelial cells. The content of NMP22 in cancerous urothelial cells is 80 times higher than that in normal cells. NMP22 in bladder cancer cells can be released into the urine in the form of cleavage fragments or complexes during cell apoptosis. The sensitivity is about 72%.

Apolipoprotein is a protein component of lipoprotein particles such as HDL and low-density lipoprotein (LDL) in serum. Apolipoproteins also have been identified in bile, with apolipoprotein A-I (Apo A-I), the predominant apolipoprotein found in HDL, found at the highest concentrations. At physiologic concentrations, Apo A-I enhanced the transfer of cholesterol from the mucosal to the serosal side of human gallbladder epithelial cell.

Apo A-I is the major protein moieties of HDL, and approximately 90% of total apo A-I and apo A-II is found within HDL density range. Apo A-I is synthesized in the liver and small intestine. It has a molecular weight of 28.000 k Da.

Exact mechanism of increased level of urinary Apo A1 level is unknown. Shin et al.[23] showed that bladder cancer with high Apo A-1 75 AA genotype reveal high level of Apo A-1 in urine. It is reported that increased urinary level of urinary Apo A-1 can facilitate diagnosis of urinary bladder cancer.[20]

Li H et al.[19] conducted a cross-sectional study titled by Identification of Apo A-1 as a biomarker for early diagnosis of bladder transitional cell carcinoma. Their study was enrolled total 72 patients among them 40 was BTCC ,24 healthy control and 8 other benign condition of urinary bladder. The sensitivity and specificity of this study was 83.7% and 89.7% respectively. The result showed significant increased APO A1 in urinary bladder cancer p value was < 0.01. This study suggest that APO A1b could be potential biomarker for diagnosis of urinary bladder cancer.

Dardeer KT et al.[16] conducted a meta-analysis titled 'Apolipoprotein A1 as a novel urinary biomarker for diagnosis of bladder cancer'. They selected total 771 patients, 417 were bladder cancer and 354 were controls. Their case control study showed sensitivity 90.7% and specificity 90% in bladder carcinoma. They showed positive ratio 9.478 and negative ratio 0.1, ROC curve was 0.9544. This study showed ApoA-1 could be a useful biomarker in diagnosis of bladder cancer.

Salem H et al.[24] performed a cross sectional study titled by Apo A1 proteins as diagnostic markers for detection of urinary bladder cancer. The study was conducted on 50 patients with carcinoma of urinary bladder, 50 patients with cystitis and 50 healthy individual. The result showed that APO A1 level significantly increased in urinary bladder cancer. The sensitivity and specificity were high. AUC was .944 on ROC curve analysis; PPV was 100%. This study revealed that APO A1 could be used as non- invasive and screening biomarker for bladder cancer.

Urinary bladder cancer is the tenth most malignant condition globally. It is the second most urothelial cancer with high recurrence and poor prognosis rate.[2] Identification of a new specific tumor marker is essential for the diagnosis of bladder cancer. This study was performed to measure urinary apolipoprotein A-1 level by Sandwiched ELISA technique. This study represents identification of a noninvasive biomarker for the diagnosis of cancer of urinary bladder.

In current study showed that the maximum number was found in the age group ≥ 53 years where 53.8% in group I and 38.5% in group II. The mean (±SD) age was 56.92±14.65 years and 38.81± 10.47 years in group I and group II respectively. Similarly, Aliramajii et al.[25] observed that mean age was 62.81±16.21 years in group I and 63.78±14.35 in group II. The difference was little higher than current study. The current study was not supported by previous study because socio economic status, life expectancy and number of study subjects of our country is a little bit lower than that country.

In this study, the gender distribution of the study subject in group I, 96.2% was male and 3.8% was female respectively. Dardee et al.[16] showed that percentage of male to female ratio of bladder cancer was 66.67 % and 33.33 % respectively. H. Salem et al.[24] showed that percentage of male to female in group I was 75% and 25% respectively. This study was not supported by those previous studies as female are discouraged to attend the male dominant health care system in our traditional mind set.

In this study, urinary apolipoprotein A-1 level was range from 27.37 to 110.28 ng/ml in group I and 3.47 to 19.24 ng/ml in group II. The mean was 47.10 ± 41.90 ng /ml and 6.21 ±7.01 ng/ml in group I and group II respectively.

In our study, cut-off value of urinary apolipoprotein A-1 showed that ≥ 20.28 ng/ml in urinary bladder cancer and less than ≤ 20.28 ng/ml in healthy group. The sensitivity and specificity were 70.83% and 92.85% respectively. Similarly, Li H et al.[19] showed that cut-off value of apolipoprotein A-1 was ≥ 18.22 ng/ml in urinary bladder cancer and ≤ 18.22 ng/ml in healthy group. The sensitivity and specificity were 83.7% and 89.7 % respectively. The result of current study was supported by previous study because detection method was similar followed by sandwiched ELISA technique.

The area under receiver operator curve characteristics (ROC) for urinary Apo A-1 level for bladder cancer was depicted by present study. The area under curve (AUC) was found 0.899. This ROC curves showed that cut off value for urinary Apo A-1 was 20.28 ng/ml which had 70.83% sensitivity and 92.85% specificity for evaluation of urinary Apo A-1 level for detection urinary bladder cancer. Li H. et al.[19] showed that cut-off value of urinary Apo A-1 was 18.22 ng/ml and AUC was 0.928. The sensitivity and specificity were 83.7% and 89.7 % respectively. Their result was consistent with present study. This study showed that urinary Apo A-1 level was significantly increased in patient with cancer of urinary bladder cancer patients. AUC suggest that urinary Apo A-1 level can be effective in predicting development of urinary bladder cancer.

Conclusion

Urinary apolipoprotein A-1 was significantly higher in patient with cancer of urinary bladder. In ROC curve analysis a cut off value of 20.28 ng/ml for urinary apolipoprotein A-1 showed highest sensitivity (70.83%) and specificity (92.85%) for predicting development of urinary bladder cancer. The area under curve (AUC) of urinary apolipoprotein A-1 level was 0.899, indicating its effectiveness in predicting development of malignancy of urinary bladder. So, urinary apolipoprotein A-1 level can be a promising biomarker in predicting development of cancer of urinary bladder.

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