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Adequacy of voided urine specimens evaluated using the Paris system for reporting urine cytology

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Abstract
Background: Adequacy (satisfactory) is a source of conflict and controversy in all cytopathology fields including the urinary tract specimens.
Aim of the study: To examine the adequacy (satisfactory) factor of urine samples and their importance in the diagnosis of urothelial malignancy using the Paris system for reporting urinary cytology.
Methods: This is a retrospective study including (314) cases of patients presented with hematuria or diagnosed as having urinary bladder mass. The slides were taken and centrifuged at 2000 RPM and stained according to the availability of the stains the year from which they obtained; Slides were taken from (2016, 2017 and 2020) were stained with (Hematoxylin and Eosin), while the slides taken from (2018-2019) were stained with (Papanicolaou) stain; In addition to some slides were stained with (Giemsa) stain in special cases, including the patients with age range of (8-98) years. All data were taken from archival files of these patients; we divide the urine volume parameter into more than and less than 30 ml.
Results: the occurrence of high grade urothelial carcinoma was encountered in 8 of 103 cases (7.8%) at age below (50) years, and 13 of 74 cases (17.6%) at age from (51-60) years, and 43 of 137 cases (31.4%) at age more than (60) years. According to the association between the urine volume and the diagnosis of high grade urothelial carcinoma, there was 19 of 68 cases (27.9%) of patient's urine volume obtained was ≤ 30 ml have high-grade urothelial carcinoma, while 45 of 246 (18.3%) of them urine volume was <30 ml have high-grade urothelial carcinoma. According to the association between adequacy of urine sample with urine volume, there were 32 of 246 (13%) of patient's urine volume obtained was <30 ml having unsatisfactory urine sample for cytological analysis, while only 6 of 68 (8.8%) of patient's urine volume obtained was ≥ 30 having unsatisfactory urine sample.
Conclusion: A significant correlation between the detection of high-grade urothelial carcinoma and increase urine volume submitted for cytological evaluation, increased urine volume submitted for cytological examination decrease the frequency of unsatisfactory samples.

Keywords: voided urine specimens, the paris system, urine cytology

Introduction

Carcinoma of bladder is usual cancer as 4th cancer occur in males occur as 3 times in males than females [1, 2]. Smoking is the utmost public danger cause that duos the danger of bladder tumor, secretarial for about 50% of the bladder tumor losses in males and 30% in females [3]. More than 90% of bladder tumors are carcinoma of urethra. [4]. There are numerous grading schemes of carcinoma of urethra but perhaps the most broadly used is that accepted by the World Health Organization (WHO). Lately, numerous examinations have been established to notice initial urinary tract tumor which includes numerous imaging models, numerous urine indicators and cystoscopy. For numerous times' urine cytology has been used to identify and survey patients with urethral carcinoma (UC) [5, 6, 7]. Doctors in general need urine cytology for all patients with UC and patients at high danger of ca. of bladder like smoking history or symptoms including "hematuria, irritative symptoms, and painful urination [8]. Asymptomatic hematuria patients must avoid urine cytology and also high risk group [9]. Cystoscopy and urothelial biopsy is a gold diagnosis for bladder cancer [9, 10, 11]. The aim of study is to examine the adequacy (satisfactory) factor of urine samples and their importance in the diagnosis of urothelial malignancy using the Paris system for reporting urinary cytology.

Method

After collection of voided urine specimens, separation of the specimen by centrifuge at 2000 RPM for fifteen minutes was done. After putting the albumen on four glass slides, the slides had been smeared and then fixed; two of the slides were fixed with ethyl alcohol 95% for 15 minutes and the other two slides stayed air-dried. After fixation the slides were already stained according to the availability of the stains in the year from which they obtained, (Hematoxylin and Eosin stain in 2016, 2017, 2020) and (PAP stain in 2018and 2019), the number of slides were stained with (H&E) was (164) and (150) slides were stained with (PAP) stain. we divide the urine volume parameter into two parameters, more than and less than 30 ml; categorize the diagnosis of the slides according to different criteria as recommended in Paris System of Urine Cytology (N\C ratio, hyperchromasia, chromatin pattern and nuclear membrane irregularity); and divide the age parameter of the patients into seven groups (0-10, 11-20, 21-30, 31-40, 41-50, 51-60; and more than 60 years). Finally, we revise the slides and applying The Paris system for reporting urine cytology to categorize the diagnosis. Statistical analysis for the results was done and assessment of correlation with different clinical and pathological parameters was measured. Papanicolaou's stain [12]: 1-Hematoxyline (Harris) for staining of the nucleus, 2-Orange (OG6) for staining of the keratin, 3-Eosin (EA65) for staining of the cytoplasm. Giemsa stain [13]: Stock Giemsa, Buffer of Giemsa, Methanol. Hematoxylin and Eosin [14]: Hematoxylin (basic dye), Eosin (acid dye). Statistical analysis was performed with SPSS v18.88 (Statistical package for social sciences) and also Excel 2010 programs. Data analysis was done using t-test, chi-square test for tables with frequencies, percentages, ranges, means standard deviation, and standard errors of the mean. Values were considered statistically significant when the p-value is equal to or less than 0.05.

Results

Cross sectional study of 314 patients, mean and SD of age (55.8 ± 17.2) years. Mean and SD of urine volume (18.3 ± 16.5) years. The number of male patients was 210 of 314(66.9%), and the number of female patients was 104 of 314 (33.1%). The number of patients have history of hematuria 179 of 314 (57%), and 135 of 314 (43%) of patients diagnosed as having urinary bladder mass, and 68 of 314 cases (21.7%) of patient's urine volume obtained was >30 ml, while 246 of 314 (78.3%) of patient's urine volume was <30 ml. As show in table (1).

Table 1: Gender, history of patients and volume size of urine distribution.

Variables	Frequency	Percentage
Gender		
Female	104	33.1
Male	210	66.9
History		
Hematuria	179	57.0
Mass	135	43.0
Volume		
< 30 ml	246	78.3
≥ 30 ml	68	21.7

As show in fig (2), (43.63%) of patients at age group > 60 years old, (23.57%) of patients at age group 51-60 years old. (11.15%) of patients at age group 41-50 years old.

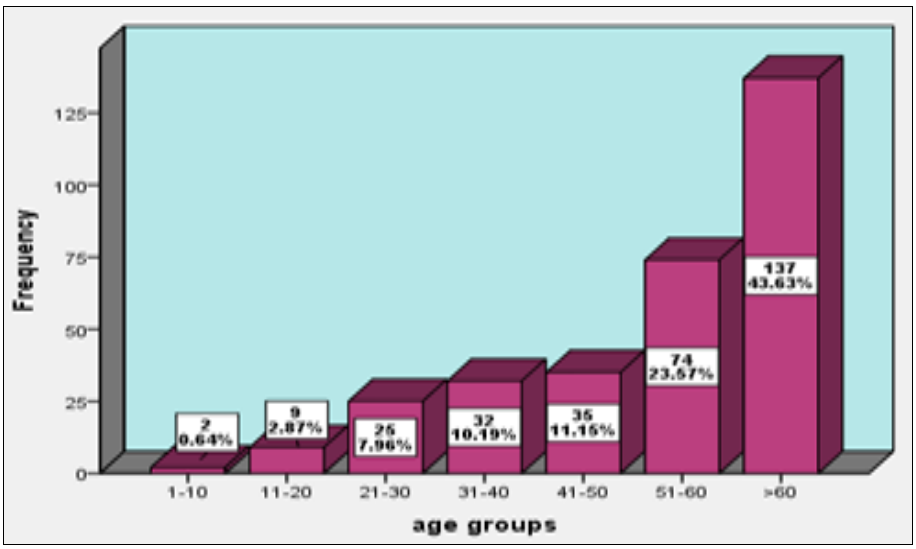

Fig 1: Age groups distribution

Histopathological diagnosis

As show in fig (1), (51.59%) of patients with Negative for high-grade urothelial carcinoma, (20.38%) of patients with High-grade urothelial carcinoma and (9.87%) of patients have Atypical urothelial cells. (12.1%) of patients have Non-diagnosis.

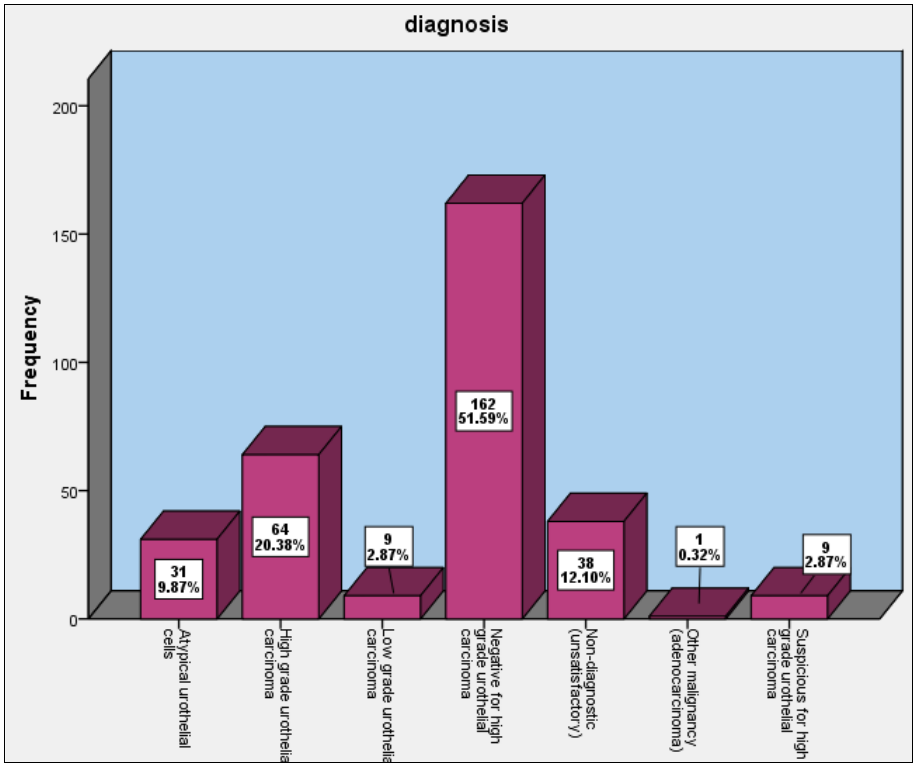

Fig 2: Cytological diagnostic criteria

Association between urine volume and gender of patients

There is no significant association between urine volume and gender of patients. As show in Table 2.

Table 2: Association between urine volume and gender of patients.

			Urine volume		P-value
			< 30 ml	≥ 30 ml	
Gender	Female	Count	86	18	
		%	35.0%	26.5%	
	Male	Count	160	50	0.24
		%	65.0%	73.5%	
Total		Count	246	68	
		%	100.0%	100.0%	

p-value ≤ 0.05 (significant)

Association between urine volume and (history, mean age) of patients

There is no significant association between urine volume and history of patients. As show in table 3**.**

Table 3: Association between urine volume and history of patients.

			Urine volume		P-value
			< 30 ml	≥ 30 ml	
History	Hematuria	Count	141	38	
		%	57.3%	55.9%	
	Mass	Count	105	30	0.89
		%	42.7%	44.1%	
Total		Count	246	68	
		%	100.0%	100.0%	

p-value ≤ 0.05 (significant)

There is no significant difference between urine volume and mean age of patients. As show in table 4**.**

Table 4: Difference between urine volume and mean age of patients.

	Urine volume	**N**	**Mean**	**Std. Deviation**	**T**	**P-value**
Age (year)	<30 ml	246	55.21	17.516	-1.327	0.19
	≥30 ml	68	58.16	15.857		

p-value ≤ 0.05 (significant)

Association between (urine volume, age groups) and cytological diagnostic criteria of patients:
There is significant association between age groups of patients and diagnosis, (88.9%) of patients at age group (11-20) years old have negative for high-grade urothelial carcinoma. (31.4%) and (17.6%) of patients at age group (>60), (41-50) years old have High-grade urothelial carcinoma respectively. As show in table 5.

Table 5: Association between age groups of patients and diagnosis.

		1-10	11-20	21-30	31-40	41-50	51-60	>60
Diagnosis	Atypical urothelial cells	0	0	1	2	1	9	18
		0.0%	0.0%	4.0%	6.3%	2.9%	1 2.2%	1 3.1%
	High grade urothelial	0	0	0	2	6	13	43
	carcinoma	0.0%	0.0%	0.0%	6.3%	1 7.1%	1 7.6%	31.4%
	Low grade urothelial	0	0	1	1	0	2	5
	carcinoma	0.0%	0.0%	4.0%	3.1%	0.0%	2.7%	3.6%
	Negative for high grade	1	8	19	23	22	36	53
	urothelial carcinoma	50.0%	88.9%	76.0%	71.9%	62.9%	48.6%	38.7%
	Non-diagnostic	1	1	3	4	4	9	16
	(unsatisfactory)	50.0%	11.1%	12.0%	12.5%	11.4%	12.2%	11.7%
	Other malignancy	0	0	0	0	0	0	1
	(adenocarcinoma)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.7%
	Suspicious for high grade	0	0	1	0	2	5	1
	urothelial carcinoma	0.0%	0.0%	4.0%	0.0%	5.7%	6.8%	0.7%
Total		2	9	25	32	35	74	137
		100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

p-value = 0.039 (significant)

There is significant association between urine volume of patients and diagnosis, (41.2%) of patients at urine volume ≥30 ml have negative for high-grade urothelial carcinoma. (27.9%) of patients at urine volume ≥30 ml have High-grade urothelial carcinoma. As show in table (6).

Table 6: Association between urine volume and cytological diagnostic criteria of patients

			Urine volume		P-value
			< 30 ml	≥ 30 ml	
Diagnosis	Atypical urothelial cells	Count	20	11	
		%	8.1%	16.2%	
	High grade urothelial carcinoma	Count	45	19	
		%	18.3%	27.9%	
	Low grade urothelial carcinoma	Count	7	2	
		%	2.8%	2.9%	0.047
	Negative for high grade urothelial carcinoma	Count	134	28	
		%	54.5%	41.2%	
	Non-diagnostic (unsatisfactory)	Count	32	6	
		%	13.0%	8.8%	
	Other malignancy (adenocarcinoma)	Count	0	1	
		%	0.0%	1.5%	
	Suspicious for high grade urothelial carcinoma	Count	8	1	
		%	3.3%	1.5%	
Total		Count	246	68	
		%	100.0%	100.0%	

p-value ≤ 0.05 (significant)

Discussion

Urothelial carcinomas of the upper urinary tract (UUT) are common (The 4th most common cancer in men and the ninth most common cancer in women in united states) [15]. Cytological examination of voided urine or washings from the UUT has been part of the standard workup for upper tract urothelial carcinoma (UTUC) (from the ureter or renal pelvis) especially it is non-invasive and easy to perform the procedure. The lack of uniform terminology and specific diagnostic criteria could also have contributed to the inferior performance of urinary cytology for detecting UTUC. The Paris System for Reporting Urinary Cytology (TPS) has provided a standardized reporting system for urinary cytology specimens with clearly defined cytomorphologic diagnostic criteria and found acceptance on an international level after its implementation in 2016. [16]. There is a significant association between age groups of patients and diagnosis of HGUC zero of 36 (0%) of patients with age group (1-30) years was diagnosed with high-grade urothelial carcinoma, while it was 2 of 32 (6.3%) of patients in age group (31-40) was diagnosed with high-grade urothelial carcinoma and it is 6 of 35 (17.1%) of age group (41-50) have HGUC. The number of patients 13 of 74 (17.6%) and 43 of 137 (31.4%) of patients in age group (51-60), (>60) years old have High-grade urothelial carcinoma, respectively. And the p-value was 0.039. That was in agreement with Onur Telli, Hasmet Sarici *et al.* (Turkey 2014). A study of 156 patients was conducted and revealed that the patients younger than 40 years with high grade urothelial carcinoma was 11of 156 (7.1%), while the incidence of high grade urothelial carcinoma was 53 of 156 (33.9%) in patients with age more than 40 years old, and

that showed a significant correlation between the incidence of high grade urothelial carcinoma and older age group with a *p*-value of 0.041 [17]. And disagree with Dionisius Alby, Abdul Hadi Hassan, Jupiter Sibarani *et al.* (Indonesia 2017). A study of 241 medical records of bladder urothelial carcinoma patients stated that the proportion of high-grade carcinoma in <65 years old age group reached 146 of 241 (60.9%), which was (27.3%) higher than in ≥65 years old age group 81 of 241 (33.6%). Chi-square test result showed a statistically significant difference between histopathological grade of urothelial carcinoma in <65 years and ≥65 years age groups (p=0.023) [18]. The cause of this disagreement is the wider range of age group that used in association with the incidence of HGUC as it used two age groups above and below 65 years and in my study I used seven age groups. In this study, the use of a 30ml cutoff value of urine volume to determine the optimum volume for the diagnosis of HGUC. There is a significant association between urine volume of patients and the diagnosis, as there was19 of 68 (27.9%) of patients at urine volume ≥30 ml have High-grade urothelial carcinoma, while 45 of 246 (18.3%) of patients at urine volume <30 ml have high-grade urothelial carcinoma, with a p-value 0.047. That was in agreement with Rezaee N, Tabatabai ZL, Olson MT. J Am *et al.* (U.S.A 2017). A study of 744 voided urine specimens. A specimen volume of ≥ 25 mL is associated with higher rates of diagnosis of HGUC or SHGUC in voided urine specimen. HGUC was diagnosed in 142 of 744 (19.1%) of specimens ≥25 ml and 100 of 744 (13.5%) of specimens <25 ml. The volume of ≥25 ml was associated with the optimum cutoff for diagnosing SHGUC or HGUC. [19]. Vanden Bussche CJ, Rosenthal DL, Olson MT *et al.* (U.S.A 2016). In total, 15,731 voided urine specimens. Specimen adequacy increased linearly for each increment of volume submitted to the laboratory up to 30 ml. Low-volume specimens below this cutoff also had lower fractions of specimens that were diagnosed as malignant or suspicious [20]. And disagree with Xing J, Qi Y, Monaco SE, Pantanowitz L. (U.S.A 2019). In our study, there is an increase in the proportion of urine that considers being adequate as it is increase in its volume. As there were 32of 246 (13.0%) of patients with urine volume <30 ml have Non-diagnostic (unsatisfactory) urine samples, while it is 6 of 68 (8.8%) of patients with urine volume ≥ 30 ml have Non-diagnostic (unsatisfactory) urine samples. p-value 0.047. That agrees with Xing J, Qi Y, Monaco SE, Pantanowitz L. There was a correlation between urine volume and the unsatisfactory/less than optimal cellularity versus satisfactory samples ($p \leq 0.001$) in voided urine specimens, but disagree regarding cutoff volume. A minimum of 10 mL of freshly voided urine was found to be a reasonable cutoff to achieve sufficient cellularity [21].

Conclusion

There is a significant correlation between the detection of high-grade urothelial carcinoma and increase urine volume submitted for cytological evaluation. Increased urine volume submitted for cytological examination decrease the frequency of unsatisfactory samples. Elderly patients with an age >60 years are at higher risk for having a high grade urothelial carcinoma.

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