

A Survey of Asymptomatic Urinary Tract Problems and Intravascular Hemolysis in Basrah Governorate

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Background: Intravascular hemolysis (IVH) is the basic pathological hallmark of a broad spectrum of diseases. Intravascular hemolysis is represented by the presence of a large amount of degradation products of red blood cells in the blood. Heme, cell-free hemoglobin and RBC micro vesicles which have promote injury of vascular and tissues by various mechanisms.

Objectives: The objective of the study is to determine the frequency rate of asymptomatic urinary tract problems and intravascular hemolysis in Basrah Governorate and to determine its association with sociodemographic, age and gender.

Methods: Cross-sectional study between July 2019 and March 2020 involved examination of 485 volunteers aged between 18-51 years old of both sex. General urine analysis was done and detection of hemosiderin in urine by using Perl's Prussian histochemical technique.

Results: Significant differences were shown on demographic, general urine analysis and intravascular hemolysis. One of the more significant findings is that relatively high incidences of asymptomatic renal problems. It was also shown that Basrah citizens have determination of presence of urine hemosiderin. Further research might investigate deposition of hemosiderin in the kidneys by using magnetic resonance imaging.

Key words: Asptomatic; UT problems; intravascular hemolysis.

1. Introduction

Intravascular hemolysis (IVH) is an assay mark of a great range of pathologies, involving socially main diseases like malaria and sickle cell disease, represented by presence of a large amount of degradation products of red blood cells (RBCs) in the blood. Heme, cell-free hemoglobin and RBC microvesicles which promote injury of vascular and tissues by various mechanisms[1]. In general, nitric oxide (NO) diminution in the microcirculation resulted from intravascular hemolysis-determined release into the plasma of cell-free hemoglobin which reacts with the vasodilator NO through the well-known deoxygenation reaction to form inert nitrate anions (NO₃⁻). Intravascular hemolysis hence reduces NO bioavailability as a result oxidative stress intensifies physiological procedures that control blood flow, angiogenesis, hemostasis and inflammation. Therefore, intravascular hemolysis represents a basic mechanism of vascular diseases[2].

Abnormal breakdown or destruction of red blood cells could be occurred in circulation system in the lumen of blood vessels (intravascular hemolysis) as a result of trauma, complement fixation or other

exogenous factors or extravascular in the body organs (extravascular hemolysis) which occur mainly in the spleen [3][4]. Intravascular hemolysis is mediated by complement system and caused by binding of anti-erythrocytes antibodies like complement-fixing IgG or IgM antibodies to the red blood cells[5], these antibodies are predominately directed against antigens that expressed on the surface of red blood cells, causing rupture of red blood cells in the blood stream and release their contents resulting in hyperbilirubinemia or icterus, decreased haptoglobin concentration and increased lactate dehydrogenase levels [6][7][8][9].

Breakdown of heme is occurred by a special enzyme that acts on heme degradation pathway and yields biliverdin, ferrous iron and carbon monoxide [10][11]. The released iron is subsequently trapped and stored either as particles (hemosiderin) in different part of the body or ferritin in the liver [12]. Once the binding capacity of circulating haptoglobin scavenger protein is exceeded in some cases of intravascular hemolysis, the plasma cell-free haemoglobin is readily filtered by the renal glomeruli. The hemoglobin is absorbed by renal proximal convoluted tubule epithelial cells and the ferric iron is stored as hemosiderin in these cells [13].

Damaged or dead renal tubular epithelial cells that containing hemosiderin granules are sloughed off from their basement membrane and excreted in the urine by urinary system, resulting in a condition called hemosiderin urea[14]. It has been observed that hemosiderin urea is associated with intravascular hemolysis (in case of defective red blood cell metabolism as in glucose-6-phosphate dehydrogenase deficiency (G6PD)), or paroxysmal nocturnal hemoglobinuria (PNH), ABO-incompatible blood transfusions and certain infectious diseases as well as severe burn injuries [3]. It is usually observed after 3-4 days of hemolysis onset, and it persists for numerous weeks after hemolysis cessation, while hemoglobin urea disappears rapidly after hemolysis cessation[15].

Hemosiderin has been identified as a marker of chronic vascular diseases; it appears in urine sediment of patients with intravascular hemolytic diseases. However, it is absent from urine of healthy subjects. Therefore, hemosiderin identifies as a new marker of chronic venous disease. Hemosiderin urine test is effective test and less costly, used to determine urinary hemosiderin[1]. Presence of hemosiderin in the urine bound to iron, the urine color appears to be "brownish", characteristically related with marked intravascular hemolysis[15].

Infections of the urinary tract (UTIs) can be with symptoms or without symptoms. Asymptomatic bacteriuria is the existence of bacteriuria without any clinical signs. While symptomatic UTI are classified as acute cystitis and pyelonephritis [16]. Unfortunately, this study was not concluded UTI study. Urinary tract infections exposed to account 22% of all infections and above 50% of prescriptions of antibiotics. Though, it's difficult to classify UTI from asymptomatic bacteriuria (ABU) on institutionalized older adults [1]. Such great ABU incidence rates clearly compromise the positive urine culture value as a diagnostic standard for UTI. Furthermore, evaluating symptoms of UTI are stimulating in this population [2].

The aim of the present work paper is to investigate the frequency rate of asymptomatic UTIs and intravascular hemolysis among individuals of Basrah Governorate and to determine its association with sociodemographic and gender.

2. Materials and methods

2.1 Selection of volunteers

Apparently healthy volunteers were selected randomly from different areas of Basrah province, southern of Iraq. Cross-sectional study between July 2019 and March 2020 involved examination of 485 volunteers aged between 18-51 years old of both sex, all of them have no previous history of clinically diagnosed renal problems, no smoking, no alcohol consumed, and no history of chronic diseases. Urine samples range from 40-50 mL was collected in sterile containers in the morning, and the tests were carried out after one hour.

2.2 general urine analyses

Random fresh urine samples usually 15 mL were obtained from healthy subjects. The sample was mixed well and centrifuged at a low speed about (2000) rpm for 15 minutes. The supernatant was emptied only 0.5 mL was left inside the tube. Then, the sediment was re-suspended through flicking the bottom of the tubes numerous times. A drop of there-suspended sediment was decanted onto a clean slide and cover slipped for microscopic urine analysis [17].

Microscopic examination of urine was performed using different magnification powers. Firstly, the slide was examined under low power (LPF) to detect crystals, casts and other large objects. Then, the examination through high power (HPF) generally used to classify cells; such as WBCs (pyuria; ≥ 5 pus cells/ μ L of urine) [18], squamous cells, crystals, and bacteria.

2.3 Determination of urine hemosiderin

Hemosiderin in urine was estimated by centrifuge 20 mL of urine at 1200g for 15 minutes, urinary sediment was used for a microscopic test. The rest of deposit was suspended by 5 mL reagent of hemosiderin staining (Perl's Prussian blue stain) then carefully mixed, allowed the sample to stand at room temperature for 10 minutes. Hemosiderin stain is prepared via mixing equal volumes from potassium ferrocyanide 2% and hydrochloric acid 2% [19]. For the second time, the tube was centrifuged and also the supernatant drew off. The sediment was re-suspended with small amount of staining liquid to wet the tube. The sample was transferred to a new microscopic slide and covered with cover slip. The slide was examined at 400x [1]. Hemosiderin granules are stained blue color by the Perl's Prussian blue stain. Isolated or grouped blue staining granules in urine smear indicate presences of hemosiderin, the test results are positive. Size of these granules is usually from (1-3) μ m [19].

3. Statistical analysis

All data from experiment were arranged and tabulated in Microsoft Excel software. Results were expressed as a percentage. Descriptive statistics has calculated for all variables across all volunteers. Statistical analysis was achieved using one-way ANOVA and also; unpaired t-test. P-value < 0.05 was considered as significant.

4. Results

4.1 Demographic estimation

The results of the demographic data are shown as documented in table 1, the frequency of age according to area. Khor Al Zubair was the greatest frequency about 38.3 ± 10.1 . Basrah Center was the lowest frequency about 28.7 ± 9 . The main age of total volunteers was 29.7 ± 9.6 .

In the present study, the age distribution of the volunteers revealed that 40 of volunteers (8.2%) were less than 20 years old, followed by 242 of volunteers (about 49.9%) were between 21-30 years old, and 112 volunteers (about 23.1%) from the total volunteers belonged to group 31-40 years old. Furthermore, 69 of volunteers (14.2%) were their ages between 41-50 years. The last age group was >50 years old; the frequency percentage was 4.5%.

The gender distribution according to areas, males (N= 203, 41.9%) of 485 total volunteers were significantly ($p < 0.001$) less than females (N= 282, 58.1%). The greatest male/female percentage was 41.9/162 in the city center, which was significant differences in number of females than male ($p < 0.001$) as illustrated in table 1

4.2 General urine examination

4.2.1 Urine pH.

The results of urine pH are shown in Table 2, only 44 of 485 volunteers (9.1% of the total number) had acidic urine, which was high significant than neutral pH ($p < 0.0001$). The high percentage was 27.3% in Abu Alkaseeb district ($P < 0.0001$). While, the lowest acidic urine percentage in AlZubair district, it was 4.8% of 63 volunteers.

4.2.2 RBC in urine.

The results, as shown in Table 2, indicate that presence of RBC in urine was about 10.7% of total 485 volunteers. In all areas included in this study, the number of samples examined that contained red blood cells in the urine was less than the number of negative samples; for example 27.3% of total 44 volunteers in Abu Alkaseeb. Furthermore, the positive results were about 6.8% of total 279 volunteers in the city center.

4.2.3 Urine pus cells.

Urinalysis performed with urine samples showed that 21.4% of total volunteers had pus cells (pyuria). Interestingly, all positive results in all studied areas were significantly differences than negative data ($p < 0.0001$). 100% of 63 tested samples were in AlZubair district ($p < 0.0001$), while the percentage of positive results in the city center was 6.5% of 279 samples (Table 2).

4.2.4 Crystals in urine

In the current study, urine crystals detected in 21.2% of total test samples (Table 2). The results showed that all collected urine samples (63) from volunteers of AlZubair district were positive (100%). However, only six volunteers (5.7%) were positive of 279 total samples in the center of Basrah.

4.3 Hemosiderin in urine

Urine hemosiderin test results from different areas of Basrah Governorate were positive in 270 (55.7%) volunteer urine samples of the total studied samples ($p < 0.01$) as illustrated in Table 3. All collected urine samples from volunteers of AlZubair district were positive (100%). While, the statistical analysis showed that the lowest percentage of urinary hemosiderin positive results was among the volunteers from Abu Alkaseeb district (31.8%).

4.3.1 Parameters of urine with positive hemosiderin samples

The differences between positive urinary hemosiderin and gender are highlighted at table 4. There were significant differences between male and female volunteers with positive hemosiderin ($p < 0.05$). From total 270 sample, there were 117(43.3%) males and 153 (56.7%) females. The greatest percentage was in AlZubair district, the females represented 74.6% of total 63 samples. However, only in Khor AlZubair district, the males were significantly higher than females; the male percentage was 73.7% of total 19 samples. From total 270 urine samples with positive hemosiderin only 31 (11.5%) of volunteers had acidic urine which significantly lower than neutral urine. The greatest percentage was in Abu Alkhasseb district, 64.3% of 14 urine samples were acidic. While the lowest percentage was in Umm Qasr district, 8.3% from 48 urine samples with positive hemosiderin were acidic.

4.3.2 RBC in Urine

show existence of hemosiderin in the urine of volunteers and presence of RBC in the urine samples. From total hemosiderin positive results (270), 56 (20.7%) of volunteers their urine contained RBC ($p < 0.0001$). The greatest percentage was in Abu AlKhasseb district, 71.4% from 14 urine samples, while the lowest results were in the center of the city, there was nine (7.1%) from 126 samples.

4.3.3 Urine pus cells

Total samples that showed positive results of hemosiderin were 270 volunteers, among 270 of volunteers who had hemosiderin positive results only 55 (20.4%) of them had pyuria (positive pus cells), which highly significant lower than negative samples ($p < 0.0001$). The greatest percentage of volunteers who had urine hemosiderin with pyuria was in Abu AlKhasseb district which 71.4% of total 14 volunteers. The lowest percentage of volunteers, who had positive urine hemosiderin and pyuria was eight (6.3%) of 126 volunteers in the center of the city as shown in.

4.3.4 Crystals in urine

Urine crystals detected in volunteers who had positive hemosiderin test results. On average, 68 (25.2%) from total hemosiderin positive samples (270) were shown to have crystals positive in their urine as in table 4. The greatest percentage of volunteers who had urine crystals with positive test hemosiderin was 100% of total 19 in Khor AlZubair district. While, the lowest positive crystals percentage was in the city center, it was about 6.3% of 126 hemosiderin positive samples.

5. Discussion

Urinary tract infections occur among both sexes, and at different ages, however the rate of incidence in females is greater than that of males, as the hormonal activity and female urethra, its closeness to the anus. Urinary tract infections are one of the most public health problems, about 10-20% of women have been reported that suffer from UTI problems during pregnancy period [20]. It is estimated that the majority of UTI cases are caused by particularly gastrointestinal bacteria [21]. Previous research has shown that 58.4% of the total examine females belonged to group of 21-30 year ages, and 26% who belonged to group at 31-40 year ages [22], the results may somewhat agree with the results of the present study. Another study conducted in Iraq, Kirkuk (29%), and Mosul (47.4%). Concerning age, the respondents distribution has shown that the age of 15–25 years was about 50.3% whereas the age of 26–35 years was 36.7% and the age of 36–45 years was 13.0% [23].

Recently, there is a study carried out on 174 healthy individuals in Nigeria, the prevalence rate of asymptomatic urinary bacteria about 28.7% of the total examine population. The study showed that the prevalence of asymptomatic urinary bacteria increased within the age range 50–59 years among

males and females. The relatively high prevalence of asymptomatic urinary bacteria is among female subjects for the same age group in comparing with males [24].

In urine, presence of blood is unusual and is related with either urinary tract problem, such as renal damage or stones, cancers, or may also be revealing of a problem of blood clotting or anticoagulant drugs undesired effects. Hematuria may be occurred due to contamination of urine with blood during the menstruation period. In young male, haematuria often occurs as a result of urine crystals formation in the urethra. Dipstick positive haematuria should be re-evaluated by urine microscopy before embarking on more extensive diagnostic investigations [17]. In a population of 1000 patients, with asymptomatic gross and microscopic hematuria, 88.3% of them had a lesion that describe hematuria and 9.1% of patients had fatal lesions, which incidence mainly in elderly and males [25].

There is a previous study reported a high prevalence of renal problems in Basrah city such as UTI and urolithiasis that developed in the last years. Crystal urea commonly exists among patients with UTI; calcium oxalate as well as uric acid. No significant differences in the incidence rate between males and females, and among all different age groups. But there was high incidence rate of uric acid crystals in both sexes than that calcium oxalates [26].

Hemoglobinuria is a prominent clinical feature due to extreme intravascular hemolysis. Once plasma hemoglobin is filtered by the glomerulus, it is reabsorbed actively by renal proximal tubule epithelial cells. Catabolism of hemoglobin in the proximal tubule leads to release of iron in the urine as hemosiderin. When the renal capacity of reabsorption is exceeded, hemoglobinuria occurs, which may lead to acute renal failure. Additionally, severe hemoglobinuria for long term is related with hemosiderin deposition on the renal proximal tubule and acute kidney failure as well as Fanconi syndrome [27].

Accumulation of hemosiderin in organs and tissues with no morphological alterations can result in hemosiderosis, which is either localized such as persistent hyperemia or haemorrhage, or generalized (systemic) such as blood transfusion and haemolytic anemia. Massive accumulation of hemosiderin in the organs and tissues is usually associated with morphological changes and functional impairment, this condition is called a hemochromatosis. Hemochromatosis is classified into primary (hereditary) type, it results from dysfunction of iron metabolism or secondary (acquired) type, this type of hemochromatosis occurs due to either frequent blood transfusions or excessive oral ingestion of iron supplements resulting in systemic changes [28].

The main finding of this study is that the positive results of the urinary hemosiderin test, 55.7% of healthy volunteer urine samples of the total studied samples had positive results. As illustrated in Table 3, the greatest percentage of urinary hemosiderin positive results was among the volunteers from AlZubiar district (100%). The positive hemosiderin results may be indicated excessive destruction of the red blood cells. A possible explanation for this might be that related to iron overload, these individuals could be received a blood for several times (repeated blood transfusion) or increase intake of iron. Another possible explanation for this is that these individuals could be exposed to war remnants or bombs during the war.

Our data showed that 56.7% of females were positive urine hemosiderin. As shows at table 4, there is a significant difference between the two groups (males and females). Hemoglobinuria is correlated with chronic urinary tract infections, especially in women who have paroxysmal nocturnal hemoglobinuria [29]. It has been found that incidence of

intracytoplasmic hemosiderin pigment within the splenic macrophages is commonly higher in female rodents than in males [30].

One of the more significant findings to emerge from this study is that determination of presence of urine hemosiderin among individuals. It also showed that Basrah citizens have relatively high incidences of asymptomatic renal problems. The findings of this study suggest that presences of renal hemosiderin may be associated with urinary tract infections. Further research might investigate deposition of hemosiderin in the kidneys by using magnetic resonance imaging.

Table 1. Showed the demographic variables in Basrah Governorate.

Demographic data				P
Criteria	Frequency: N (%)			value
Age	29.7 ± 9.6			<0.05 consider significant
Age according to Area				
City center	28.7 ± 9			
AbuAlKhaseeb	31.3 ± 7.8			
Umm Qasr	31.3 ± 10.5			
AlZubair	29.7 ± 9.7			
Khor AlZubair	38.3 ± 10.1			
Age distribution				
≤20	40(8.2%)			
21-30	242(49.9%)			
31-40	112(23.1%)			
41-50	69(14.2%)			
>50	22(4.5%)			
Gender distribution according to area				Table 2. shows how
	N	male	female	
City center	279	117(41.9%)	162(58.1%)	
Abu AlKhaseeb	44	22(50%)	22(50%)	
Umm Qasr	73	24(32.9%)	49(67.1%)	
AlZubair	63	20(31.7%)	43(68.3%)	
Khor AlZubair	26	20(76.9%)	6(23.1%)	
Total	485	203(41.9%)	282(58.1%)	

d Parameters of general urine examination.

Urine pH				
Area	N	Neutral	Acidic	P values
City center	279	259(92.8%)	20(7.2%)	0.0001
Abu AlKhaseeb	44	32(72.7%)	12(27.3%)	
Umm Qasr	73	69(94.5%)	4(5.5%)	
AlZubair	63	60(95.2%)	3(4.8%)	
KhorAlZubair	26	21(80.8%)	5(19.2%)	
Total	485	441(90.9%)	44(9.1%)	<0.0001
RBC in urine				
Area	N	+ve	-ve	

City center	279	19(6.8%)	260(93.2%)	
Abu AlKhaseeb	44	12(27.3%)	32(72.7%)	
Umm Qasr	73	6(8.2%)	67(91.8%)	<0.001
AlZubair	63	10(15.9%)	53(84.1%)	
Khor AlZubair	26	5(19.2%)	21(80.8%)	
Total	485	52(10.7%)	433(89.3%)	<0.0001
Pus in urine				
City center	279	18(6.5%)	261(93.5%)	
Abu AlKhaseeb	44	12(27.3%)	32(72.7%)	
Umm Qasr	73	6(8.2%)	67(91.8%)	<0.0001
AlZubair	63	63(100%)	0(0%)	
Khor AlZubair	26	5(19.2%)	21(80.8%)	
Total	485	104(21.4%)	381(78.6%)	<0.0001
Crystals in urine				
City center	279	16 (5.7%)	263 (94.3%)	
Abu AlKhaseeb	44	10 (22.7%)	34 (77.3%)	
Umm Qasr	73	9 (12.3%)	64 (87.7%)	<0.0001
AlZubair	63	63 (100%)	0 (0%)	
Khor AlZubair	26	5 (19.2%)	21 (80.8%)	
Total	485	103 (21.2%)	382 (78.8%)	<0.0001

P value <0.05 consider significant

Table 3.Results of hemosiderin test in urine according to including areas.

Hemosiderin in urine				
Area	N	+ve	-ve	P value
City center	279	126 (45.2%)	153 (54.8%)	
Abu AlKhaseeb	44	14 (31.8%)	30 (68.2%)	
Umm Qasr	73	48 (65.8%)	25 (34.2%)	<0.0001
AlZubair	63	63 (100%)	0 (0%)	
Khor AlZubair	26	19 (73.1%)	7 (26.9%)	
Total	485	270 (55.7%)	215 (44.3%)	<0.01

P value <0.05 consider significant

Table 4. showed the Parameters of urine analysis with hemosiderin positive results.

Area	N	Gender		P values
		Male	Female	
City centre	126	63(50%)	63(50%)	
Abu AlKhaseeb	14	5(35.7%)	9(64.3%)	
Umm Qasr	48	19(39.6%)	29(60.4%)	<0.001
AlZubair	63	16(25.4%)	47(74.6%)	
Khor AlZubair	19	14(73.7%)	5(26.3%)	
Total	270	117(43.3%)	153(56.7%)	<0.05
Area	N	Urine pH		P values
		Acidic	Neutral	
City centre	126	11(8.7%)	115(91.3%)	
Abu AlKhaseeb	14	9(64.3%)	5(35.7%)	<0.0001
Umm Qasr	48	4(8.3%)	44(91.7%)	

AlZubair	63	3(4.8%)	60(95.2%)	
Khor AlZubair	19	4(21.1%)	15(78.9%)	
Total	270	31(11.5%)	239(88.5%)	<0.0001
RBC in urine				
Area	N	+ve	-ve	
City centre	126	9(7.1%)	117(92.9%)	
Abu AlKhaseeb	14	10(71.4%)	4(28.6%)	<0.0001
Umm Qasr	48	6(12.5%)	42(87.5%)	
AlZubair	63	27(42.9%)	36(57.1%)	
Khor AlZubair	19	4(21.1%)	15(78.9%)	
Total	270	56(20.7%)	214(79.3%)	<0.0001
Pus in urine				
City centre	126	8(6.3%)	118(93.7%)	
Abu AlKhaseeb	14	10(71.4%)	4(28.6%)	<0.0001
Umm Qasr	48	8(16.7%)	40(83.3%)	1
AlZubair	63	25(39.7%)	38(60.3%)	
Khor AlZubair	19	4(21.1%)	15(78.9%)	
Total	270	55(20.4%)	215(79.6%)	<0.0001
Crystals in urine				
Area	N	+ve	-ve	P value
City centre	126	8 (6.3%)	118 (93.7%)	
Abu AlKhaseeb	14	9 (64.3%)	5 (35.7%)	<0.0001
Umm Qasr	48	7 (14.6%)	41 (85.4%)	1
AlZubair	63	25 (39.7%)	38 (60.3%)	
Khor AlZubair	19	19 (100%)	0 (0%)	
Total	270	68 (25.2%)	202 (74.8%)	<0.0001

P value <0.05 consider significant

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