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Evaluation of Antioxidant Capacity And Ascorbic Acid In Children With Urinary Tract Infection

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ABSTRACT: Background: Urinary tract infection (UTI) is one of the most common pediatric infections. It causes oxidative stress and leads to suppression of the antioxidant enzymes.

Aim: To assess the association between total antioxidant capacity, ascorbic acid status and urinary tract infection in children.

Material and Methods: This case control study included 30 pediatric patients with urinary tract infection documented by urine culture and sensitivity. Their ages ranged from 6:12 years, they were selected from children attending the outpatient pediatric clinic and inpatients of the pediatric department, Al-Zahraa University hospital during the period from March 2015 to December 2015. Also it included 30 healthy children age and sex matched as a control group. Assessment of serum ascorbic acid and total antioxidant capacity were done for both groups.

Results: There was significant decrease in serum total antioxidant capacity levels in cases compared to the controls, it was (319.96 ± 53.10mmol/L)) in cases and (549.74±57.69 mmol/L) in the controls, (P <0.01), also there was significant decrease in ascorbic acid serum levels in cases compared to the control group, it was (0.25±0.07 mmol/L) and (0.50±0.06 mmol/L) respectively with (P <0.01). Positive correlation between serum total antioxidant capacity and vitamin C status (r = 0.767; p<0.001). Significant positive correlation between ascorbic acid serum level and RBCs (r = 0.466; p < 0.001).

Conclusion: Antioxidants and Ascorbic status may influence susceptibility to urinary tract infection in children and can be used as an adjuvant in treatment of UTI as it help body to overcome oxidative stress caused by infection and restore its healthy environment.

Key words: *Urinary tract infection, total antioxidant, ascorbic acid.*

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I. INTRODUCTION

Urinary tract infection in children are more likely to be serious than those in adults and should not be ignored (especially in younger children). Urinary tract infection is second only to respiratory infection as the most common type of infection ***(Shaikh et al., 2013)[1].***

Early and appropriate diagnosis of UTI in infants and young children is important as it is marker for urinary tract problems such asrenal scarring and reflux nephropathy which can lead to hypertension and end stage renal failure ***(Saeed et al., 2015)[2].***

Oxidative stress as a state of imbalance between oxidant and antioxidant's processes of the body may be both the cause and the consequence of many diseases. Nowadays, it is a well-known that oxidative stress plays an important role in pathogenesis of various kidney diseases such as UTI. The kidney is an organ highly vulnerable to damage caused by reactive oxygen species due to abundance of long chain polyunsaturated fatty acids in the composition of renal lipid. Due to this fact the determination of the capacity of the antioxidant defense may be important for improving the health of children ***(Ozbek, 2012)[3].***

Ascorbic acid (vitamin C) is one molecule which has proven preventive functions against the stresses. It is a powerful antioxidant that defends the cells from oxidative damage occurring due to aerobic metabolism and a biotic stress ***(Upadhyaya et al., 2010)[4].***

The relationship between vitamin C intake and lower urinary tract symptoms is of interest because of the benefits of reduced systemic inflammation and oxidative stress for the lower urinary tract structure and function ***(Buscemi et al., 2012)[5].***

We aimed to investigate total antioxidant capacity and ascorbic acid status in children with UTI and compare them with controls.

Subjects and Methods

This case control study was carried out on 30 children with urinary tract infection confirmed by: Pyuria (pus cell ≥10/ HPF) in urine sample collected under sterile condition and +ve urine culture ***(Habib , 2012)***[6], during the period from March 2015 to December 2015 , these are the patients numbers who attended at the pediatric outpatient clinic during the period

of the study with documented urine culture and full fill the inclusion criteria and also who agreed to complete the study **.** Also 30 healthy children with matched age and sex were enrolled as a control group. Informed consent was obtained from the participating parents in adherence with the guidelines of the ethical committee of Alzhraa hospital, AL-Azhar University, Cairo, Egypt. Children (with infections of other systems, chronic illness, malnourishment, urologic disease or congenital renal abnormalities and children with antioxidant and vitamin C therapy were excluded from the study. All patients and controls were subjected to: full history taking, full clinical examination, laboratory and radiological investigations. Informed consent was obtained from the participating parents in adherence with the guidelines of the ethical committee of Alzhraa hospital, AL-Azhar University, Cairo, Egypt.

Blood sampling:

- 6 ml were withdrawn by sterile venepuncture.
- 2 ml were added to EDTA vacutainer for CBC.
- 2 ml were allowed to clot then separate the serum by centrifugation at room temperature. The separated serum was used to measure BUN, creatinine and CRP.
- 2ml were taken in heparinized tubes and immediately stored on ice at 4°C then plasma separated from the cells by centrifugation at 3000 rpm for 10 min at -4°C and the plasma samples were stored at -80 °C till the time of assay for total antioxidant capacity and ascorbic acid serum levels.

Laboratory investigations:

Routine investigations

Urine analysis, urine culture and sensitivity,on a clean catch specimen, more than 10000 colonies in boys suggest likely infection and 100000 colonies in girls makes the diagnosis of an infection likely ***(Chishti et al., 2010)***[7]**.** Complete blood picture, BUN and serum creatinine.

Specific investigations:

1- Assessment of total antioxidant capacity in the serum of all children's study using method of ***(Miller et al., 1993)***[8]**.**

The assay was based on the principle that ABTS (2,2-Azino-di-[3-ethylbenzthiazoline sulphonate]) is incubated with a peroxidase and H_2O_2 to produce the radical cation ABTS+. This has a relatively stable blue green colour, which is measured at 600 nm. Antioxidant in the samples suppresses this colour production to a degree which is proportional to their concentration. The assay results are expressed as umolTroloxEq/l.

2- Assessment of ascorbic acid in the serum of all children's study using enzyme linked ferric tripyridyltriazine spectrophotometric assay (EFTSA) ***(Benzie, 1996)***[9]**.**

Radiological investigations:

Pelvi-abdominal ultrasound: to investigate urologic disease or congenital abnormalities but also can give substantive evidence for renal parenchymal disease.

II. STATISTICAL ANALYSIS

Data were collected, revised, coded and entered to the statistical package for social science (SPSS) version (16). Comparison between two groups with qualitative data was done by using Chi-square test while the comparison between two independent groups with quantitative data and parametric distribution were done by using independent t-test. Spearman correlation was used to assess the correlation between two quantitative parameters in the same group. Receiver operating characteristic curve (ROC) was used to assess the best cut off point with its accuracy, sensitivity, specificity, PPV and NPV. The confidence interval was set to 95% and the margin of error accepted was set to 5%. So the p-value was considered non-significantat $P > 0.05$ and significant at $P < 0.05$.

III. RESULTS

Table 1 shows comparison between children with UTI and healthy controls regarding demographic data, anthropometric measurements and blood pressure, it revealed: no significant difference between both groups.

Table 2 shows comparison between children with UTI and healthy controls regarding laboratory data, it revealed: significant increase in WBCs and decrease in Hb level, total anti-oxidant capacity and ascorbic acid serum levels in children with UTI compared to healthy controls. Also the same table shows that out of 30 children with UTI ,25 patients have positive CRP level.

Figure 1 shows the prevalence of lower and upper UTI in the study cases, it was,25 (83.33%) and 5 (16.66%) respectively.

Figure 2 shows that 60% of the study cases were found with recurrent attack of UTI while 40% were with first attack.

Table 3 shows correlation between antioxidant capacity and some of the studied parameters, it revealed: significant positive correlation between antioxidant capacity and ascorbic acid serum level.

Figure 3 shows significant positive correlation between ascorbic acid and RBCs.

Table 4 figure 4 show the cut off point, sensitivity and specificity of total anti-oxidant capacity and ascorbic as a risk for UTI in children, it revealed that: sensitivity, 100% specificity, 100% PPV and 96.8% NPV, while the cut off value of ascorbic acid serum level was < 0.393 to predict UTI with 100% sensitivity, 100% specificity, 100% PPV and 100% NPV.

Table (1): Comparison between patients and controls regarding clinical data

Variable		**Control group No. 30**	**Patients group No. 30**	**Chi-square test**	
		Mean ±SD	Mean ±SD	**x^2/t***	**p-value**
Age (years)		8.87±2.64	8.87±2.64	0.000*	1.000
Weight(kg)		30.47±9.14	29.90±8.26	0.252	0.252
Height(cm)		131.40±17.00	128.83±14.58	0.628	0.533
BMI		17.10±1.04	16.88±0.92	0.888	0.378
Systolic (mm Hg)		100.50±8.74	98.67±7.42	0.876	0.385
Diastolic (mm Hg)		66.33±4.72	64.50±4.97	1.464	0.149
Sex	Female	26 (86.70%)	26 (86.70%)	0.000	1.000
	Male	4 (13.30%)	4 (13.30%)		

Table (2): Comparison between patients and the controls regarding laboratory data

Variable		**Control No.30**	**Patients No.30**	**Independent t-test**	
		Mean± SD	**Mean± SD**	**x^2/t**	**p-value**
WBCs (x10^3/ul)		6.36 ± 1.36	7.67 ± 1.27	3.844	0.001**
RBCs (x10^6/ul)		4.79 ± 0.25	4.77 0±41	-0.190	0.850
Hb(g /dl)		12.40 ± 0.37	11.69 ± 0.49	-6.374	0.001**
Platelets (x10^3/ul)		369.52 ±134.33	392.32 ± 30.43	0.828	0.412
BUN(mg /dl)		24.32 ±7.28	24.00 ± 4.25	0.190	0.850
Creat. (mg /dl)		0.54 ± 0.05	0.50 ± 0.10	-1.960	0.055
CRP	Negative	30 (100.0%)	5 (16.7%)	42.857	0.001
	Positive	Positive	25 (83.3%)		
Total antioxidant capacity(mmol/L)		549.74 ± 57.69	319.96 ± 53.10	16.052	0.001**
Vitamin C (mmol/L)		0.50 ± 0.06	0.25±0.07	14.388	0.001**

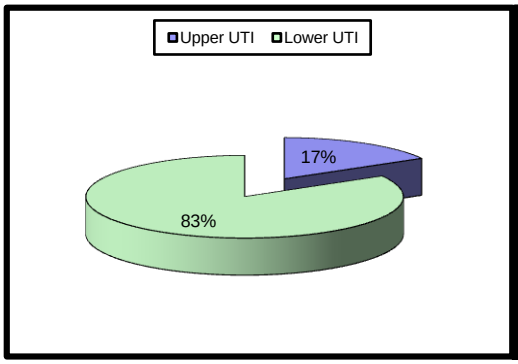

Figure (1): Prevalence rate of upper and lower UTI in patients group.

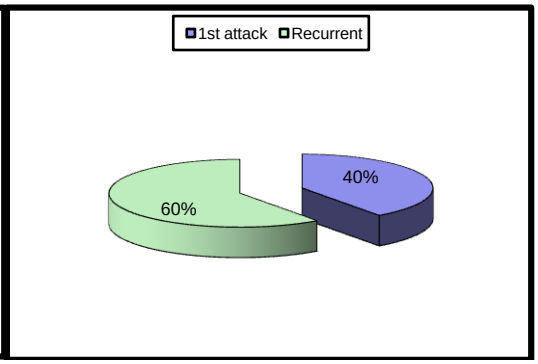

Figure (2): The recurrence rate of UTI in patients group**.**

Table (3): Correlation between total antioxidant capacity with clinical and laboratory findings

Variable	Total antioxidant capacity	
	r	p-value
Age/ys	0.338	0.068
Weight (kg)	0.273	0.144
Height (cm)	0.329	0.076
BMI	0.301	0.120
Systolic (mm Hg)	0.138	0.468
Diastolic (mm Hg)	0.279	0.136
WBCs (x10^3/ul)	0.094	0.620
RBCs (x10^6/ul)	0.195	0.303
Hb(g/dl)	0.025	0.897
ESR (normal <10)	0.088	0.646
CRP	-0.040	0.834
BUN (mg/dl)	-0.284	0.128
Creatinine(mg/dl)	-0.032	0.866
Vitamin c (mmol/L)	0.767	0.001

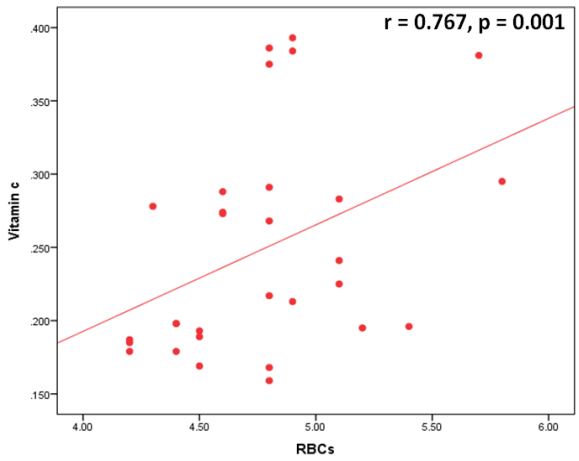

Figure (3): Correlation between serum ascorbic acid and RBCs.

Table (4): Cut off point, AUC, sensitivity, specificity, PPV and NPV for TAC and vitamin C in prediction of UTI

Variable	Cut off point	AUC	Sensitivity	Specificity	PPV	NPV
TAC (mmol/L)	≤402.8	99.9%	96.7%	100%	100.0%	96.8%
VitaminC(mmol/L)	≤0.393	100%	100%	100%	100%	100%

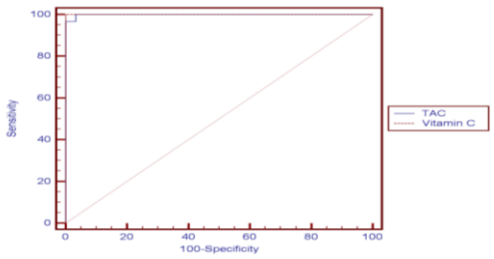

Figure (4): ROC curve between patients and the controls regarding TAC and vitamin C serum levels.

IV. DISCUSSION

Antioxidants are our crucial defense against free radical induced damage and are critical for maintaining optimum health and wellbeing. Nowadays, it is a well-known that oxidative stress plays an important role in pathogenesis of various kidney diseases such as UTI ***(Ozbek, 2012)*[3].**According to literature survey our study represents the first analysis of the interaction between antioxidant capacity, vitamin C serum levels and children with UTI. Other studies reported the relation between antioxidant capacity and vitamin C status in patients (either children or adults) with other diseases. Our finding showed significant decrease in total antioxidant capacity and ascorbic acid serum levels in cases compared to the control group. Lower levels of them in UTI cases may reflect its importance in immunity thus infection. Increased formation of ROS and/or decreased antioxidant defense can be defined as oxidative stress, which is widely recognized as an important feature of many diseases. Ascorbate is considered to be the most important low molecular-weight water-soluble antioxidant in plasma, based on both its concentration and its reaction rate with physiologically relevant oxidants ***(Halliwell, 2012)*[10].**

Foxman and Chi (1990)[11] found that vitamin C gives protection against UTI. Ascorbic acid is delivered to tissues and leukocytes, probably due to increased consumption in the cell and high leukocyte turnover. Since intracellular ascorbate concentrations higher than in plasma, a high production and turnover of these cells contributes to depletion. Low plasma concentrations correlate with inflammation***(Burzle et al., 2013)*[12].**

In the same line with our results ,**Ciftci et al**. 2008[13]reported similar finding but it was an adult study .

Ciragil et al. (2005)[14], found in 164 urine samples obtained from patients with pre-diagnosis of acute UTI, determined the effects of oxidative stress in UTI and concluded that UTI causes oxidative stress, increases lipid peroxidation level and leads to insufficiency of antioxidant enzymes.

Previous longitudinal follow-up Boston Area Community Health survey data support the inverse association for dietary vitamin C, as citrus (orange or grapefruit) juice was inversely associated with progression of LUTS and urgency symptoms over time ***(Curto et al.,2014)*[15].**

Also ***Fowler et al. (2014)***[16] reported that vitamin C levels are subnormal in multiple human inflammatory disease states including :sepsis, systemic inflammatory response syndrome (SIRS), trauma, and cancer.

In disagreement with our study**, *Birder et al. (2010)*[17]** who studied asymptomatic female adult volunteers, greater urine acidity leads to greater micturition desire and urgency thus, a relatively immediate effect of high dose vitamin C on exacerbating LUTS.In general, vitamin C is adequately stored in the body with 75 mg/day, and>200 mg/day is likely to be excreted through urine **Levine etal. (1995)**[18].Urine composition and pH have been shown to affect theurothelium, resulting in changes in afferent nerve activity and underlying smooth muscle **Birder**etal.(2010)[17]

In a study of asymptomaticfemale volunteers, greater urine acidity led to greater micturition desire and urgency **Lavin**etal. (1997)[19], thus, a relatively immediate effect of high dose vitamin C on exacerbating LUTS is plausible.It is possible that the observed associations between dietary vitamin C and LUTS were not attributable to vitamin C itself, these patient with age may have urine storage problems and other different associations that must be investigated .

***Villanueva and Kross (2012)*[20]**showed the controversy around dietary antioxidants ,because the capacity to display antioxidant and pro-oxidant behavior depends on various factors, this contradict the statement, endogenous and exogenous antioxidants act as "free radical scavengers" by preventing and repairing damages caused by ROS; and therefore can enhance the immune defense and lower the risk of disease ***(Gupta et al., 2012)*[21].**

Interestingly we found that there was significant positive correlation between vitamin C serum level and RBCs. Vitamin C is one of antioxidants which antagonize oxidative stress and protect RBCs from oxidative damage ***(Lang et al., 2006)*[22]**.

By the way, regarding the recurrence rate of UTI among the studied cases ,60% experienced recurrent UTI calculated during their long life and documented with urine culture and sensitivity , with no significant correlation to ascorbic acid and total anti-oxidant capacity .It was not included in the aim but it may reflect the prognosis of the disease, also ***Hooton (2012)*[23]**reported similar finding, while ***Conway et al. (2011)*[24]** showed that 83 among 611 cases with UTI aged 6 years or younger were with recurrent UTI, so the recurrent rate was 13.6% the difference may attributed to age difference and sample size**.**Interestingly by ROC curve analysis for both TAC and ascorbic acid status, both rise to the rank of ideal markers for prediction of UTI, this findings are in need for further researches as there is no available data according to the comprehensive electronic survey.

In conclusion, low total antioxidant capacity and ascorbic acid serum levels may influence susceptibility to urinary tract infection in children. Antioxidants and ascorbic acid can be used as an adjuvant

drug in treatment of UTI as new drugs and in foods and medicinal materials within the recommended daily dose to overcome oxidative stress caused by infection to restore healthy environment.

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