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EC MICROBIOLOGY
Research Protocol

Role and Clinical Significance of Routine Urine Analysis in Prediction of Covid-19 Disease Severity

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Received: September 10, 2020; **Published:** May 31, 2021

Abstract

Introduction: COVID-19 patients usually present with flu-like symptoms however some undergo rapid deterioration and even death. Although acute respiratory failure is the main documented feature, involvement of other organs still needs to be explored.

Aims: Our aim was to assess the importance of urine biochemical parameters with the help of automated urine analyser in the prediction of disease severity in COVID-19 cases.

Settings and Design: A single centre study was conducted which included 170 symptomatic patients with confirmed COVID-19 infection.

Materials and Methods: The clinical parameters of these patients were studied from the hospital records. Each patient's biochemical and flowcytometric examination of the urine samples was done using the Sysmex UX 2000 analyser. Various urine parameters were compared between the ICU and isolation ward group.

Statistical Analysis Used: Statistical analysis was done by student t test.

Results: The COVID-19 patients in ICU had significantly higher urine glucose ($p = 0.021$), protein ($p = 0.002$), urobilinogen ($p < 0.001$), ketone ($p < 0.001$), blood ($p < 0.001$), LEU ($p < 0.001$), RBC ($p = 0.001$) and WBC levels ($p < 0.001$) in comparison to isolation ward patients. On subgroup analysis excluding comorbidities, the result remained the same except the value of specific gravity ($p = 0.034$) which was significantly higher in ICU cases. The patients with comorbidities who were treated in ICU had a significantly higher urobilinogen ($p = 0.039$), WBC level ($p = 0.003$) and urine ketone ($p = 0.003$).

Conclusion: The differences in glucose, protein, blood, RBC, specific gravity and LEU are significant between the patients in ICU and isolation ward which is not associated with comorbidities. Hence, these indicators can be used for the assessment of severity among COVID-19 patients.

Keywords: *SARS-CoV-2; Urine Analysis; Urine Biochemical Parameters; Intensive Care Unit; Comorbidities*

Abbreviations

IW: Isolation Ward; ICU: Intensive Care Unit; LEU: Leucocyte Esterase; RBC: Red Blood Cell; WBC Levels: White Blood Cell Levels; SRC: Small Round Cells; EC: Epithelial Cell; CRS: Cytokine Release Syndrome; ACE 2 receptor: Acetyl Coenzyme 2 Receptor; SARS: Severe Acute Respiratory Syndrome; UTIs: Urinary Tract Infections; PRO: Proteinuria; pH: Potential of Hydrogen; SG: Specific Gravity; KET: Ketone; GLU-U: Urine Glucose; BLOOD: Urine Occult Blood; DM: Diabetes Mellitus; HTN: Hypertension

Introduction

In December 2019, an outbreak of cluster of cases of pneumonia of an unknown etiology was reported from Wuhan city, the capital of Hubei province of China. A novel coronavirus SARS-CoV-2 was isolated from these patients with virus infected pneumonia, which later was designated as coronavirus disease 2019 (COVID-19) by WHO (World Health Organization) in February 2020 [1]. COVID-19 outbreak has been declared a global emergency by WHO. As the number of cases continue to rise, it is clear that this virus is posing a great threat to public health. Detailed clinical as well as virological course of this illness have not been well described till date.

COVID-19 patients usually present with flu-like symptoms, amongst which some undergo rapid deterioration and few even death [2,3]. Most of the times the patients show features of lung injury with complications in other organs such as liver, heart as well as gastrointestinal tract especially in severe cases [4]. Cytokine release syndrome (CRS) or cytokine storm which includes systemic endothelial injury has been well documented in COVID-19 cases [5]. The underlying mechanism is studied as the ACE2 receptor, whose expression has been reported in the alveolar cells in the lungs, brush border of proximal tubular cells as well as podocytes in kidney, heart and the gut [6]. It acts as a cell entry receptor thus causing interferon-gamma-related cytokine storm, leading to multiple organ injuries in SARS patients [7,8]. Diffuse alveolar damage and acute respiratory failure although are the main documented features of COVID-19, the involvement of other organs still needs to be explored. In recent studies on COVID-19 patients, an influence on kidney function was also found in certain patient groups, including acute kidney damage and proteinuria [9].

Initially urine examination was done for the auxiliary diagnosis of urinary tract infections (UTIs) in COVID-19 patients but later studies have reported impaired urine routine parameters in COVID-19 patients [10-13]. Automated urine analysers based on principles of flow cytometry and cytochemistry have revolutionized the reporting in laboratory medicine. Not only it aids in quick and efficient reporting of routine urine samples, the results also are precise and accurate. Flow cytometry based automated urine analysers in use nowadays have reduced the urine screening time to mere minutes. Automated urine flow cytometry based analysers can detect particles in urine like WBC, RBC, crystals and bacteria while at the same time use chemical strips to detect concentration of various components present in urine [14].

Routine urine examination is considered as a quick, convenient and economical test to detect biochemical parameters that can be used for auxiliary diagnosis of UTIs, early diagnosis of kidney impairment and monitoring the treatment effects in COVID-19 patient. There is very limited information on kidney involvement as well as about the correlation between urine biochemical parameters and COVID-19 disease.

Aim of the Study

The aim of this study was to explore the biochemical as well as microscopic urine parameters that may predict the severity of the disease in COVID -19 patients.

Materials and Methods

Study design: Total 170 symptomatic patients with confirmed COVID-19 infection were enrolled in this study from 7th April 2020 to 4th June 2020. They were admitted in ICU or isolation ward for further management as per the severity of the presenting symptoms. Asymptomatic patients or suspected COVID-19 patients whose results were awaited were not included in the current study.

Sample collection and interpretation: Midstream urine samples of the diagnosed positive patients were collected in a sterile urine container. Each sample underwent biochemical and flowcytometric examination using the Sysmex UX 2000 (Sysmex Corporation, Japan), a fully automated urine analyser. The UX 2000 aspirates 2.2 ml of urine; 0.95ml for cytochemistry (CHM) and 1.2 ml for flow cytometry (FCM). UX2000 uses transmission refractrometry to detect specific gravity, light scattering for determining turbidity and reflectivity for colour detection. CHM analyses test strips uses dual wavelength reflectance method. The test strips are coated with the urine sample and it reads after 60 seconds [14]. For flow cytometry, the aspirated sample is stained with fluorescent dye and analysed in two channels and categorizes the particles in urine into RBC, WBC, epithelial cells, cast and bacteria using a classification algorithm [10]. In this study, we evaluated all the parameters, with special emphasis on the biochemical parameters which includes urine occult blood (BLOOD), proteinuria (PRO), bilirubin, urobilinogen, potential of hydrogen (pH), specific gravity (SG), ketone (KET), urine glucose (GLU-U) and leukocyte-esterase (LEU) as well as flowcytometric parameters which includes red blood cells (RBC), white blood cells (WBC) and small round cells (SRC).

The clinical parameters of the patients were studied retrospectively from the stored hospital records which included age, sex, the severity of the symptoms and the associated comorbidities if any. The patients included in comorbid group had history of one or more than one of the following diseases like diabetes mellitus, hypertension, thyroid disease, coronary artery disease, known or operated malignancy, chronic obstructed pulmonary disease and chronic liver disease.

Statistical analysis: The statistical analysis was performed using Microsoft Excel (Microsoft Corporation, Washington, USA) as well as Statistical Package for the Social Sciences (IBM, Chicago, USA). Normally distributed measurement data were expressed as the mean SD and were analysed using student t-test between two samples. A p-value of < 0.05 indicates statistical significant values.

Results

Total 170 patients were enrolled in the present study with age ranging from 9 to 96 years. Among all patients, 128 (75.2%) were males and 42 (24.7%) were females. The median age for male was 48 years (9 - 96 years) and female was 51 years (21 - 79 years). The male: female ratio was 3:1. Further, based on clinical presentation, 126 patients were admitted in isolation ward, among which 84 patients were males (median age: 44 years; range: 9 - 96 years) and 26 patients were females (median age: 52 years; range: 19 - 68 years). Remaining 44 severe cases were admitted in intensive care unit, among which 33 patients were males (median age: 56 years; range: 30 - 82 years) and 11 patients were females (median age: 48 years; range: 38 - 79 years).

Of all total cases, 151 patients were not having any coexisting comorbidity and 19 patients were suffering from diabetes and/or hypertension. We analysed the urine routine microscopy findings of all the patients treated.

Urine biochemical parameters in diagnosed patients of Covid-19 among ICU and isolation patients

On analyzing the urine routine microscopy of the patients treated in ICU versus isolation wards we noticed that patients in ICU had significantly higher urine glucose ($p = 0.021$), protein ($p = 0.002$), urobilinogen ($p < 0.001$), ketone ($p < 0.001$), blood ($p < 0.001$), LEU ($p < 0.001$), RBC ($p = 0.001$) and WBC levels ($p < 0.001$). pH, specific gravity, EC and SRC were similar in both the groups (Table 1).

	RICU		Isolation Ward		
	Mean	SD	Mean	2SD	p value
Glucose	191.6	378	110	280	0.021
Protein	97.7	89.8	41.5	64.4	0.002
Urobilinogen	1.7	2.2	0.71	1.3	<0.001
pH	6.3	0.7	6.3	0.7	0.768
Ketone	10.23	30	1.8	13.9	<0.001
Blood	0.11	0.3	0.01	0.04	<0.001
LEU	70.5	155.4	23.6	89.7	<0.001
Specific gravity	1.021	0.009	1.017	0.01	0.276
RBC	863.1	5344	33.4	180.1	0.001
WBC	117.5	331.9	21.7	46.7	<0.001

Table 1: *Urine biochemical parameters in diagnosed patients of COVID-19 among all ICU and isolation patients.*

Urine biochemical parameters in Covid-19 patients without comorbidities in ICU and isolation ward

We considered coexisting diabetes and hypertension as a confounding factor in the analysis and did a subgroup analysis of the patients who did not have any history of HTN. On subgroup analysis (total n = 151, n = 38 (ICU), n = 113 (IW)), we noticed that patients who were treated in ICU had a significantly higher urine glucose ($p = 0.001$), protein ($p = 0.013$), urobilinogen ($p < 0.005$), ketone ($p < 0.001$), blood ($p < 0.001$), LEU ($p < 0.001$), specific gravity ($p = 0.034$), RBC ($p < 0.001$) and WBC level ($p < 0.001$). pH, EC and SRC were similar in both the groups (Table 2).

Non - Comorbidities					
	RICU		Isolation Ward		
	Mean	SD	Mean	SD	p value
Glucose	165.6	352.6	74.3	224.4	0.001
Protein	107	90.4	48.1	82.1	0.013
Urobilinogen	1.5	2.1	0.74	1.4	0.005
pH	6.4	0.7	6.3	0.7	0.931
Ketone	8.4	23.8	1.9	14.5	< 0.001
Blood	0.16	0.27	0.02	0.05	< 0.001
LEU	103.5	173.6	26.6	93.3	< 0.001
Specific gravity	1.0201	0.009	1.016	0.01	0.034
RBC	1505.8	7011.5	56.9	256.6	< 0.001
WBC	190.9	424.4	23.6	48.2	< 0.001

Table 2: *Urine biochemical parameters in COVID-19 patients without comorbidities in ICU and isolation ward.*

Urine biochemical parameters in Covid-19 patients with associated comorbidities admitted in ICU and isolation wards

The COVID -19 patients with associated comorbidities who were treated in ICU had a significantly higher urobilinogen ($p = 0.039$), WBC level ($p = 0.003$) and urine ketone ($p = 0.031$) than those treated in isolation wards (Table 3).

Comorbidities					
	RICU		Isolation Ward		
	Mean	SD	Mean	SD	p value
Glucose	203.3	464.6	384.6	477.6	0.325
Protein	71.7	31.9	40.8	60.5	0.287
Urobilinogen	1.5	1.8	0.5	1.1	0.039
pH	5.9	0.5	6.2	0.7	0.613
Ketone	25	61.2	2.3	6	0.003
Blood	0.005	0.01	0.15	0.05	0.32
Specific gravity	1.025	0.007	1.017	0.009	0.131
RBC	28.9	12.8	12.6	12.4	0.838
WBC	22.5	33.4	11.9	9.1	0.031

Table 3: *Urine biochemical parameters in COVID-19 patients with associated comorbidities admitted in ICU and isolation wards.*

Discussion

The outbreak of COVID-19 had started in China at the end of 2019 and since then this pandemic has spread its terror globally leading to a large number of deaths worldwide. COVID-19 was determined to be caused by SARS-CoV-2 through an RNA-based meta-genomic next-generation sequencing approach [15]. Most of the COVID-19 patients are asymptomatic; however few of them are symptomatic with usual clinical symptoms that are fever, cough, myalgia and diarrhoea [16,17]. Older patients present with severe Older patients present with severe symptoms are more likely to have respiratory failure are more likely to have respiratory failure and rapid disease progression to multiple organ dysfunctions [18].

Urine is considered frequent biometrics for discovery of parameters that can detect early warning signs and are sensitive for disease detection because of their accessibility; non-invasiveness and can genuinely reflect the changes of the body health conditions [19].

The team of experts from the University Medical Center Goettingen noticed that in severely ill patients of COVID-19; in addition to lungs and heart, kidneys are also badly affected. Urine analysis exhibited low antithrombin III concentrations, severe hypoalbuminemia and positive results for blood, albumin and leukocytes in urine of COVID-19 patients [20]. A recent article by Haifeng Zhou., *et al*. demonstrated that COVID-19 patient with abnormalities in routine urine examination such as proteinuria, haematuria and leucocytouria can present with early renal-impairment [12]. Another prospective cohort study on COVID-19 patients admitted in a tertiary teaching hospital at Wuhan showed that among 701 patients, 43% presented with proteinuria, 11% with haematuria and 3.5% to 5% with acute kidney injury and was associated with increased mortality [21].

In our study, most of our patients presented with proteinuria, glucosuria, ketonuria, urobilinuria and haematuria as well as positive results for RBC, WBC and LEU in routine urine examination. The value was significantly higher when compared between ICU and isolation ward patients irrespective of status of co-morbidities. Other parameters did not show substantial difference. On subgroup analysis, considering only patients without co-morbidities, the result remained the same except the value of specific gravity which was significantly higher in ICU cases when compared with isolation ward cases. These findings were similar to a study published in a Chinese cohort [22] as well as an Italian cohort [23].

In severely ill patients, ARDS is the most common presentation as SARS-CoV-2 infection that may trigger cytokine storm which leads to multiple organ dysfuction syndrome (MODS) including kidney [24,25]. Therefore, renal injury caused by cytokine storm could be responsible for the presence of glucosuria and proteinuria in COVID-19 patients.

However, urinary ketone was significantly higher in our study which goes against other studies which concluded that temporary hyperglycemia did not cause the patient's tendency toward ketoacidosis [22].

On subgroup analysis, considering COVID-19 patients with associated comorbidities, these patients showed significantly higher urobilinogen, WBC levels and urine ketones when compared between ICU and isolation ward patients.

Hence, the results indicate that the differences in glucose, protein, blood, RBC, specific gravity and LEU are caused by SARS-CoV-2 infection and not due to the presence of comorbidities. These indicators can be used for the assessment of severity among COVID-19 patients as well as prediction of the complications.

Even though this study is single centre study and we have included patients with confirmed COVID-19 disease, still we believe that routine urine examination is a rapid, cheap, easy and an excellent method to assess the progression of disease and predict the severity of infection in COVID-19 patients. Studies with larger sample size would be required for further evaluation and assessment of the significant urine parameters in COVID-19 patients.

Conclusion

The differences in glucose, protein, blood, RBC, specific gravity and LEU are significantly different between the patients in ICU and isolation ward and are not associated with comorbidities. Hence, awareness regarding abnormal urinalysis in COVID-19 patients should be increased as screening of the urine biochemical parameters can detect potential kidney impairment which a clinician should keep in mind while treating these patients. Routine urine examination is a quick, convenient, economical and easy tool to detect biochemical parameters that can be used for assessment and predicting progression of disease in severely infected COVID-19 patients making it an additional armamentarium for early detection and effective treatment of renal involvement in COVID-19 cases.

Acknowledgements

We would like to acknowledge all our laboratory technicians and support staff for their overwhelming dedication during these hard times.

Conflict of Interest

There are no conflicts of interest.

Bibliography

1. Wang Chen., *et al.* "A novel coronavirus outbreak of global health concern". *The Lancet* 395.10223 (2020): 470-473.

2. X Yang., *et al.* "Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study". *The Lancet Respiratory Medicine* 8 (2020): 475-481.

3. Wang D., *et al.* "Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China". *The Journal of the American Medical Association* 323.11 (2020): 1061-1069.

4. Zaim S., *et al.* "COVID-19 and Multiorgan Response". *Current Problems in Cardiology* 45.8 (2020): 100618.

5. Picchianti Diamanti A., *et al.* "Cytokine Release Syndrome in COVID-19 Patients, A New Scenario for an Old Concern: The Fragile Balance between Infections and Autoimmunity". *International Journal of Molecular Sciences* 21.9 (2020): 3330.

6. H Zhang., *et al.* "Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target". *Intensive Care Medicine* 46.4 (2020): 586-590.

7. Hoffmann M., *et al.* "SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor". *Cell* E8 (2020): 271-280.

8. Huang KJ., *et al.* "An interferon-gamma-related cytokine storm in SARS patients". *Journal of Medical Virology* 75.2 (2005): 185-194.

9. Yang X., *et al.* "Prevalence and impact of acute renal impairment on COVID-19: a systematic review and meta-analysis". *Critical Care* 24 (2020): 356.

10. Falbo R., *et al.* "Bacteriuria screening by automated whole-field image-based microscopy reduces the number of urine cultures". *Journal of Clinical Microbiology* 50 (2012): 1427-1429.

11. Erdman P., *et al.* "The accuracy of the Sysmex UF-1000i in urine bacterial detection compared with the standard urine analysis and culture". *Archives of Pathology and Laboratory Medicine* 141 (2017): 1540-1543.

12. Haifeng Zhou., *et al.* "Urinalysis, but not blood biochemistry, detects the early renal-impairment in patients with COVID-19". *Med Rxiv* (2020).

13. Liu R., *et al.* "The value of urine biochemical parameters in the prediction of the severity of coronavirus disease 2019". *Clinical Chemistry and Laboratory Medicine* 58.7 (2020): 1121-1124.

14. Berger RE. "The urine dipstick test useful to rule out infections. A meta-analysis of the accuracy". *The Journal of Urology* 174 (2005): 941-942.

15. Chen L., *et al.* "RNA based mNGS approach identifies a novel human coronavirus from two individual pneumonia cases in 2019 Wuhan outbreak". *Emerging Microbes and Infections* 9 (2020): 313-319.

16. Guan WJ., *et al.* "Clinical characteristics of 2019 novel coronavirus infection in China". *The New England Journal of Medicine* 382 (2020): 1708-1720.

17. Chan JF., *et al.* "A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster". *Lancet* 395 (2020): 514-523.

18. Huang C., *et al.* "Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China". *Lancet* 395 (2020): 497-506.

19. Zhao M., *et al.* "A comprehensive analysis and annotation of human normal urinary proteome". *Scientific Reports* 7.1 (2017): 3024.

20. Gross O., *et al.* "COVID-19-associated nephritis: early warning for disease severity and complications?". *Lancet* 395.10236 (2020): e87-e88.

21. Cheng Y., *et al.* "Kidney disease is associated with in-hospital death of patients with COVID-19". *Kidney International* (2020).

22. Liu R., *et al.* "The value of urine biochemical parameters in the prediction of the severity of coronavirus disease 2019"0. *Clinical Chemistry and Laboratory Medicine (CCLM)* 58.7 (2020): 1121-1124.

23. Bonetti G., *et al.* "Urinalysis parameters for predicting severity in coronavirus disease 2019 (COVID-19)". *Clinical Chemistry and Laboratory Medicine (CCLM)* (2020): 20200576.

24. Guan WJ., *et al.* "Clinical characteristics of 2019 novel coronavirus infection in China". *The New England Journal of Medicine* (2020).

25. Chan JF., *et al.* "A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster". *Lancet* 395 (2020): 514-523.

Volume 17 Issue 6 June 2021