Study of urine parameters and presence of virus in covid-19 patients

BY

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"STUDY OF URINE PARAMETERS AND PRESENCE OF VIRUS IN THE URINE IN COVID-19 PATIENTS"

DOCTOR OF MEDICINE

IN

PATHOLOGY

LIST OF ABBREVIATIONS

ABBREVIATION	PARAMETER
ACE2	Angiotensin-converting enzyme 2
SARS-CoV-2	Severe acute respiratory syndrome
MERS-CoV	Middle East Respiratory Syndrome Coronavirus
RT-PCR	Reverse Transcriptase - Polymerase Chain Reaction
Ct	Cycle threshold
PPE	Personal protective equipment
HRCT	High-resolution computed tomography
pH	Potential hydrogen

ABSTRACT

INTRODUCTION

COVID-19 infection caused by the SARS-COV2 virus was responsible for the pandemic. COVID-19 is transmitted by respiratory droplets and direct contact, although viral shedding in urine was also possible. The clinical significance of urine analysis for predicting the severity of coronavirus disease has also been described. To better manage COVID-19 patients, this study evaluated the presence of the virus and changes in parameters in the urine samples from COVID-19 patients.

OBJECTIVES

The study was to evaluate the presence of the virus and the changes in urine biochemical parameters of COVID-19 patients to know the clinical significance for better management of the disease.

METHODS

A hospital-based cross-sectional study was carried out on all patients admitted in Covid wards in BLDE(Deemed to be University) Shri B. M. Patil Medical College, Hospital and Research Centre, Vijayapura. Urine samples of RT-PCR-positive patients who are non-diabetic, non-hypertensive and with no underlying renal disease of all age groups admitted in the covid ward were collected. We used a LAURA SMART urine analyzer to determine the urine parameters, and the RT-PCR method was MAVERICK'S NUCLEIC ACID EXTRACTION KIT for urine D.N.A. extraction.

RESULTS

Out of 50 COVID-19-positive patients, proteinuria, glucosuria, haematuria and ketonuria were seen in COVID-19 positive patients. Seven of these patients showed severe HRCT scores, implying a correlation between biochemical parameters with their severity. There was increase

in proteinuria and glucosuria in patients with positive urine RT-PCR results than in patients who were tested negative for urine RT-PCR. Five cases(10%) out of 50 were positive for urine RT-PCR, and one patient died of more severe disease.

CONCLUSION

COVID-19 patients manifests with proteinuria, glucosuria, haematuria and ketonuria. These patients might signify its impact on the kidney and severe COVID-19 Infection can lead to severe kidney effects and subsequent renal failure. Furthermore, the SARS-COV-2 virus in urine indicates that covid-19 patients can shed the virus in the urine. So, clinicians and pathologists should be aware of these consequences for successfully further disease management and also be aware that transmission of the disease through urine is possible.

KEYWORDS

SARS-COV2, URINE RT-PCR, URINE BIOCHEMICAL PARAMETERS.

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"STUDY OF URINE PARAMETERS AND PRESENCE OF VIRUS IN THE URINE IN COVID-19 PATIENTS."

INTRODUCTION

The severe acute respiratory distress syndrome coronavirus (SARS-CoV-2) was discovered in Wuhan, China and spread globally, and was declared a pandemic on March 11th, 2020. The SARS-CoV-2 virus primarily causes acute respiratory illness, but multiple organs can also be affected.

Novel Coronavirus manifests as acute respiratory illness, primarily affecting the lungs in the individuals. In severe cases, it causes death due to ARDS and pneumonia. However, reports have also shown that many organs, including the kidney, are involved[2]. Pathophysiology of coronavirus disease 2019 is still under research[3]. Some authors established that urinary tract involvement in COVID-19 is one of the common manifestations, and the gradual deterioration of renal function is an adverse prognostic factor [4].

The study on urine analysis can help medical science to research the exact cause of this situation further. Only a few studies are present regarding the prediction of severity with abnormal urine biochemical parameters and the status of the SARS-COV-2 virus in the urine. Hence the present study was conducted to evaluate the changes.

Inhalation of virus particles and personal contact are the two most common ways Coronavirus disease 2019 spreads from person to person. However, the virus can be shed in urine, and the infection can spread through contaminated urine. Based on the viral genome's similarity to the coronavirus disease 2019 and earlier proof of the virus's presence in the urine, it was possible to transmit the virus through urine[5].

However, it is unknown how viruses are shed through urine. In the urine, there appear to be two mechanisms for Coronavirus disease 2019 shedding: Firstly, sepsis and the cytokines storm cause renal dysfunction and consequently, Coronavirus disease 2019 is released into the urine; and secondly, the virus may adhere to ACE2 receptors and shed into the urine, directly invading the urinary system. It is possible that viruses could shed into the urine, but most studies indicate that infected patients have no virus in their urine[6,7-8].

Despite this, the data about specific characteristics of coronavirus disease 2019 is limited. Urinary sediment microscopy is a reliable diagnostic tool and a prognostic indicator. Urine specimens were collected and handled with Personal Protection Equipment kit(PPE) to perform MicrExUrSed under microscopy.

Current research on the presence of SARS-CoV-2 in urine in humans has produced conflicting results, as several studies with large samples of urine failed to detect any traces of the virus using either the Real-Time Reverse Transcriptase-Polymerase Chain Reaction method or the nucleic acid amplification method.

However, the Real-Time Polymerase Chain Reaction technique in this investigation detected SARS-CoV-2 in the urine of more than 10% of the 50 COVID-19-positive patients.

OBJECTIVES OF THE STUDY

1. To study the urine parameters in COVID-19-positive patients and to correlate its values with the severity of the disease.

2. To know the prevalence of positivity of the virus in the urine in COVID-19-positive patients.

REVIEW OF LITERATURE

HISTORY

Coronavirus Disease-2019 (COVID-19) has been attributed to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and resulted in many deaths[9]. Researchers have investigated SARS-CoV-2, the host's response to it, and its epidemiological and clinical characteristics.

As of March 11th, 2020, the World Health Organization (WHO) declared a pandemic due to the severe acute respiratory syndrome coronavirus 2 (COVID-19) outbreak in China. With the advent of severe acute respiratory syndrome (SARS) in 2002 and Middle East respiratory syndrome (MERS) in 2012, scientific developments have enabled researchers to understand the pathology and epidemiology of SARS-CoV-2 better and develop effective treatments.

Elderly patients with comorbidities such as diabetes, obesity, lung diseases and heart diseases predominantly suffer from severe symptoms of COVID-19. There has been evidence that SARS-CoV-2 causes damage to the lung, kidneys and other vital organs where the ACE2 gene is highly expressed. In general, COVID-19 patients experience mild symptoms and recover without or with minimal medical attention[10]. Without immediate oxygen therapy and mechanical ventilation, many patients with respiratory distress symptoms died rapidly[11].

It is, therefore, imperative to develop novel approaches to estimate disease stages so patients can seek appropriate treatments and allocate scarce medical resources correctly. To understand SARS-CoV-2 pathogenesis, novel detection methods that understand the underlying changes in molecular and biological processes of COVID-19 patients are also needed. Urine and blood are frequent biomaterials for discovering biomarkers of human diseases because of their accessibility and non-invasiveness.

VIROLOGY

Origin and Genome-

Initially, the SARS-CoV-2 virus was isolated from the bronchoalveolar lavage fluid of three COVID-19 patients in China. The SARS-CoV-2 viruses are enveloped, positive-sense single-stranded RNA viruses and α, β, γ, and δ-CoVs are four subtypes classified serologically. α- and β-CoVs causes human CoV infections[12]. SARS-CoV-2 shows 80% and 45% sequence identity to SARS-CoV and MERS-CoV, respectively, according to the genetic analysis.

The size of the viral genome is 29-32 kb approximately. The viral replication and transcription complex consists of structural, accessory, and non-structural proteins encoded by the gene. It comprises genomic RNA and nucleocapsid (N) protein. Glycoprotein spikes appear on the membrane and surround the genome, which is enclosed in a nucleocapsid that is helical in relaxed form and assumes a spherical shape in the virus particle. It has phospholipid bilayers, and two spike

proteins covering SARS-CoV-2 are the spike glycoprotein(S) and the hemagglutinin-esterase (HE). In the viral envelope, the membrane protein (M) and the envelope protein (E) are found among the S proteins[13].

Viruses are capable of infecting different cells primarily because of the spike glycoprotein. This protein has two subunits, a subunit called S1 that binds to angiotensin-converting enzyme 2 (ACE2) and a subunit called S2 that facilitates membrane fusion. Transmembrane serine protease binds to ACE2 on the target cell(TMPRSS2) and cleaves the spike protein at the S2′ site. Virus-host lipid bilayers are fused by this cleavage, allowing the viral ribonucleoprotein complex to enter the cell. As a result of these structural proteins combining with a lipid bilayer taken from the host, the virus particle is formed, which delivers viral genomic RNA to the cells[14]. The SARS-CoV-2 genome has 5′ and 3′ terminal sequences, typical of β-CoVs.

During natural infection in humans, the targeted cells by SARS-CoV-2 are in the nasal olfactory mucosa or the nasopharynx. In the presence of its positive-sense genome, SARS-CoV-2 produces proteins, including replication factories, that are located in the endoplasmic reticulum. It is through the regulation of interferon-stimulated genes that interferons indirectly or directly promote an antiviral state. In addition, cytokines aid in the development of an immune system that helps eliminate the virus.

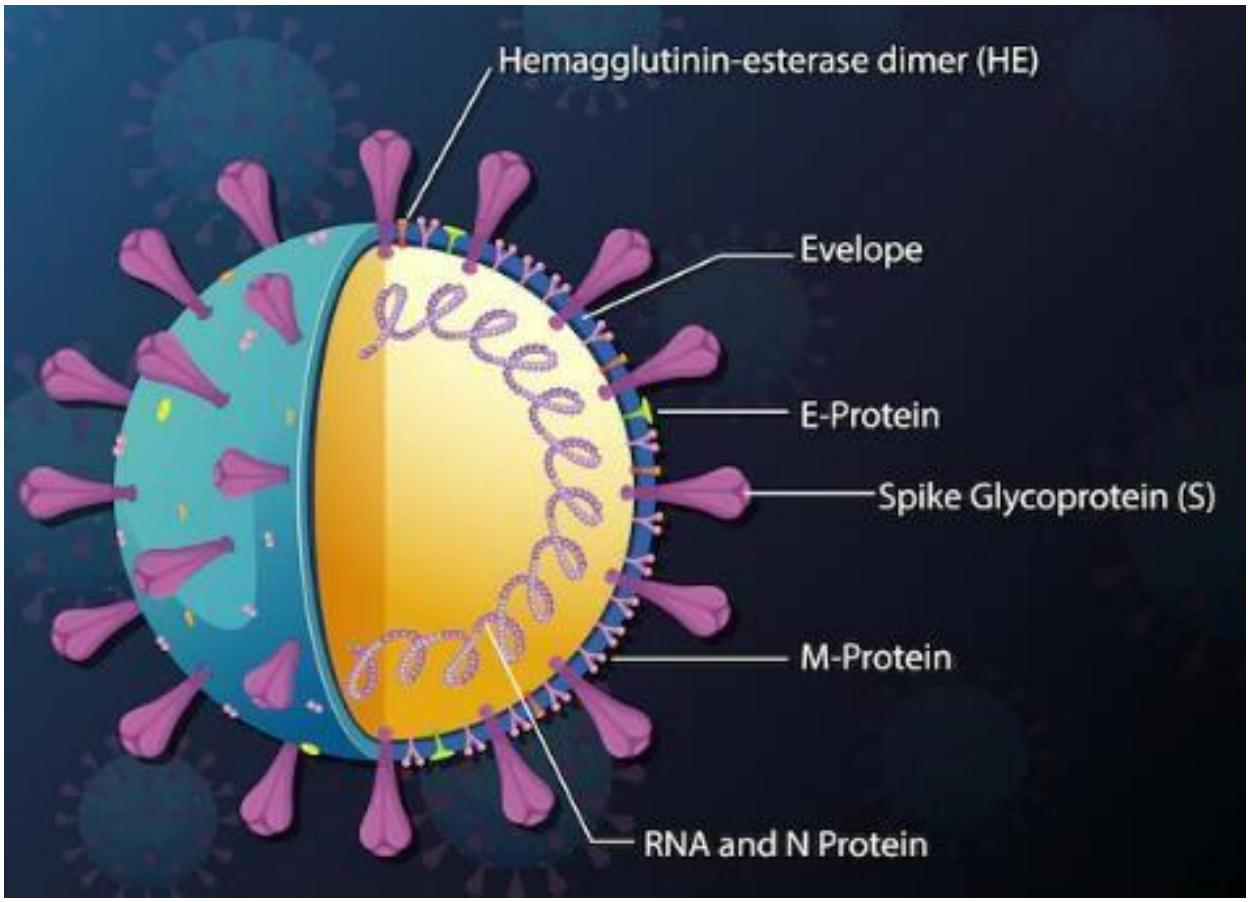
It is possible for the virus to spread to the lower respiratory tract through inhalation of virus particles if the immune system fails to respond to it as a result of the weak immune response. Lower respiratory tract infections may result in inflammation and reduced gas exchange in the alveoli. Alveolar type II cells in the lungs' alveoli are primarily infected by the virus, which secretes surfactants that lubricate the lungs.

Endothelial activation can result in direct alveolar damage caused by infection of alveolar type II cells. Damage-associated transient progenitors (DATP) are AT-II cells that fail to differentiate fully and are associated with lung injury. Fluid leaks into the alveoli due to ruptured epithelium and endothelium. Endothelial injury exposes the subendothelial extracellular matrix(ECM), facilitating platelet activation and coagulation cascade. Activated complement leads to T cell activation that damages microvascular endothelial cells and releases chemokines. Thereby, further lung damage hampers oxygen exchange and causes hypoxaemia or ARDS.

Genomic variations-

Several variations may exist among the SARS-CoV-2 genomes obtained from COVID-19 patients. However, Tang et al. reported, based on genomic analyses of 103 samples, that SARS-CoV-2 evolved into two major types of L and S. L type types may be more aggressive and spread more quickly due to intense selective pressure[15,16]. SARS-CoV-2 R.N.A. viruses are extremely unstable,

making continuous surveillance from humans or animals essential to control the disease.



Viral serotypes-

Coronaviruses are zoonotic, involving birds and mammals, with bats hosting an enormous variety of genotypes. Six non-SARS coronavirus serotypes have been associated with disease in humans. Human-to-human transmission occurs mainly through respiratory droplets and contact routes.

Viral shedding-

Viral shedding in the respiratory tract is seen 1-2 days before the onset of symptoms, persists for 7-12 days and up to 2 weeks in severe cases. In urine and faeces, viral shedding is seen five days after the onset of symptoms and up to 4-5 weeks. Viral RNA shedding may not equate with infectivity.

Receptor Interactions and Cell Entry-

SARS-CoV-2 mediates cell entry through the ACE2 receptor. The ACE2 is expressed mainly in the lungs, heart, kidney, and intestine and heart diseases. It has a C-terminal domain and an N-terminal domain. ACE2 cleavs angiotensin (Ang) I to produce angiotensin. Additionally, ACE2 is a direct binding site for proteins belonging to the CoV-S family. S proteins of CoV-S exist, undergo structural rearrangements and fuse with host cell membranes.

EPIDEMIOLOGY:

The spectrum of Infection-

COVID-19 usually exhibits its symptoms after a 5-day incubation period. It may also vary with the age of the patient and the patient's immune system. The symptoms vary from asymptomatic to clinical illness characterized by acute respiratory distress syndrome leading to pneumonia and multi-organ dysfunction[,18].

The typical clinical manifestations of patients with COVID-19 are fever, dry cough, rhinorrhoea, sneezing, sore throat, and fatigue. COVID-19 patients are most commonly present with breathlessness, which results from hypoxaemia. Soon after shortness of breath and hypoxaemia start, progressive respiratory failure occurs, leading to ARDS. More than half of patients are dyspnoeic. Typical findings of chest CT images were ground-glass opacity and areas of consolidation.

Source of Infection-

It is transmitted mainly through respiratory droplets and aerosols. Patients with COVID-19 are the most common sources of Infection, and severe cases are more contagious than mild cases. People who are asymptomatically infected or those in incubation, despite showing no signs or symptoms of respiratory Infection, may also be potential sources of Infection. Infection is higher within 7 to 10 days after infection when viral RNA levels from upper respiratory specimens are at their peak, and the infectious virus is most likely to be detectable. Viral RNA detected for a prolonged period does not indicate prolonged infection. There appears to be a threshold of viral RNA level below which infectiousness is unlikely.

In a few studies, the RT-PCR test showed continuous positive results for samples from patients who recovered from COVID-19.

Routes of Transmission-

Respiratory droplets and contact transmission are the main transmission routes. Direct person-to-person respiratory transmission is the primary mode of transmission of COVID-19. Few studies reported the presence of the virus in urine, stool, blood, and semen samples, signifying that faecal-oral transmission was less likely, and few studies are reported.

Herd susceptibility-

Older adults and persons with underlying primary disorders such as hypertension, diabetes, heart diseases and renal disorders may be more susceptible to SARS-CoV-2. Obesity and smoking are also the predisposing factors[14].

High-Risk Population-

Increased age and obesity are the predisposing factors, and the common comorbidities include hypertension, heart disease, diabetes, kidney failure and chronic lung diseases.

Persons in close contact with patients or sub-clinically symptomatic infected persons are part of the high-risk population. High infection risk is also taken into account by healthcare professionals and patients' families.

DIAGNOSIS

Nucleic Acid Test-

COVID-19 Infection is diagnosed using RT-PCR, next-generation sequencing, and nucleic acid detection kits[16]. Samples from nasopharyngeal, oropharyngeal swabs, urine, bronchoalveolar lavage (BAL), and saliva were used to detect SARS-CoV-2 nucleic acid. The specimen adequacy, the collection method and the duration since exposure are some variables that affect PCR testing's sensitivity.

SARS-CoV-2 antigen assays are less sensitive than molecular PCR testing, although they have a faster turnaround time.

Complete blood counts(CBC), Chest X-rays, Computed Tomography(CT) scan, Erythrocyte sedimentation ratio(ESR) and C-reactive protein (CRP) are other diagnostic modalities.

HRCT chest is a non-invasive, cost-effective, rapid and reliable diagnostic tool that is not only superior in diagnosing COVID-19 but also prompt and commonly available. A non-invasive, affordable, fast, and reliable diagnostic method called HRCT chest is an excellent diagnostic tool for detecting COVID-19. Elevated HRCT score is an independent prognostic factor of mortality risk in severe COVID-19 patients.

Based on CT severity scores, COVID-19 infection is classified as Mild: <9, Moderate: 9-15 and Severe: >15.

Serologic Diagnosis-

Various serological responses are demonstrated in patients of coronavirus disease 2019. Appropriate detection reagents have been developed quickly in conjunction with other technologies like immunochromatography, colloidal gold, and others.

PATHOGENESIS:

SARS-CoV-2 invades host cell-

Following viral transmission, ACE 2 protein is highly expressed in multiple human cells, including type II alveolar cells, urothelial cells of the bladder, proximal tubule cells of the kidneys, and myocardial cells, mediating the entry of SARS-CoV2. There have been three pathogenic stages of COVID-19 suggested for SARS in literature: viral replication, activating the immune system, and causing lung damage[17].

It was suggested that COVID-19 has the following clinical phases: viremia, acute, and recovery phase[18]. The virus preferably penetrates host cells, replicates, assembles, and then releases extracellularly to infect target cells, which destroys alveolar epithelial cells. ARDS, sepsis, and MODS are caused by the simultaneous release of many pathogen-associated molecular patterns (PAMP) and damage-associated molecular pattern (DAMP) molecules. They activate the

innate immune system, cause inflammatory cell infiltration, and release cytokines, chemokines, proteases, and free radicals.

Renin-angiotensin system in COVID-19-

ACE2 is converts AT-II into AT-1. The S- protein of SARS-CoV2 recognizes the host ACE2 receptor and facilitates viral entry. In addition to facilitating viral entry, absence of Angiotensin-converting enzyme 2 activity has been linked to acute lung damage[19] because loss of ACE2 can result in renin-angiotensin system (RAS) failure, which can worsen inflammation and increased vascular permeability. In SARS-CoV patients, one confusing issue is that majority manage to eliminate the infection despite the inflammatory reactions. but a few who produce neutralizing antibodies early, develop persistent inflammation, ARDS, or even sudden death. The same mechanism is also seen in SARS-CoV-2 infection.

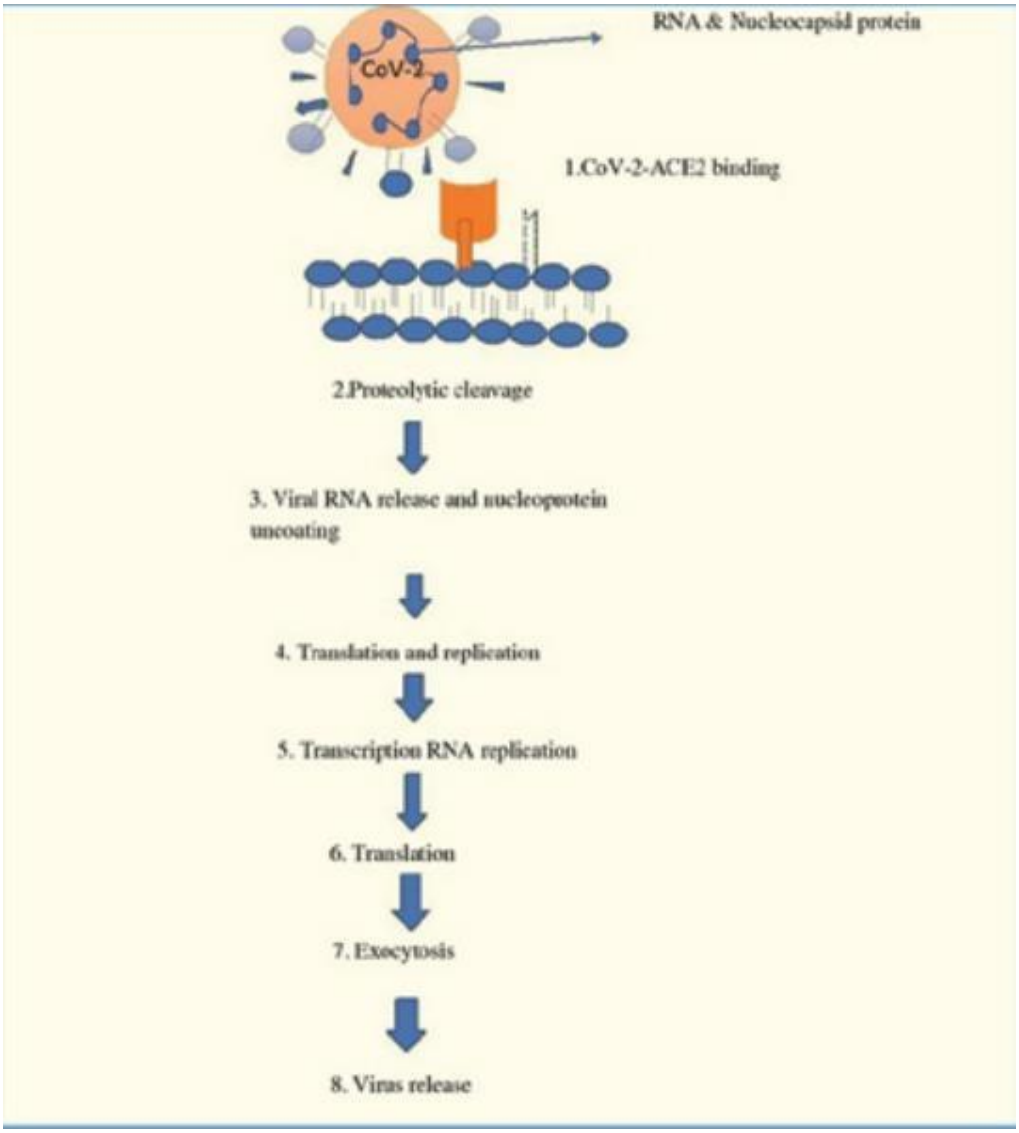
Direct cytopathic effect of SARS-CoV-2-

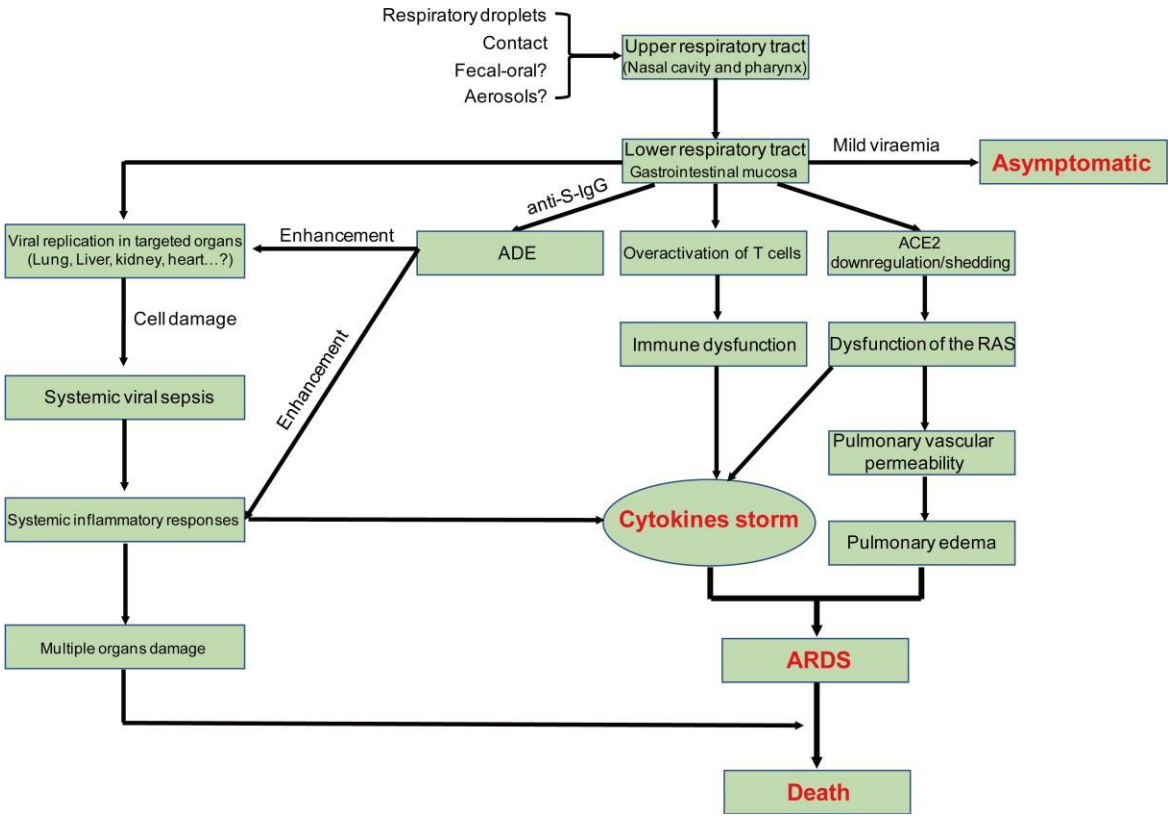
The findings of COVID-19-induced lung infection are similar to those in SARS-CoV and MERS-CoV infections, including bilateral alveolar destruction, patchy inflammatory cellular infiltration, and intra-alveolar oedema. It is believed that SARS-CoV-2 is responsible for the significant pathological alterations in vital organs associated with COVID-19, both directly and indirectly through its cytopathic effects or abnormal immune response respectively. There is some indication that an abnormal immune response (rather than a direct viral cytopathic

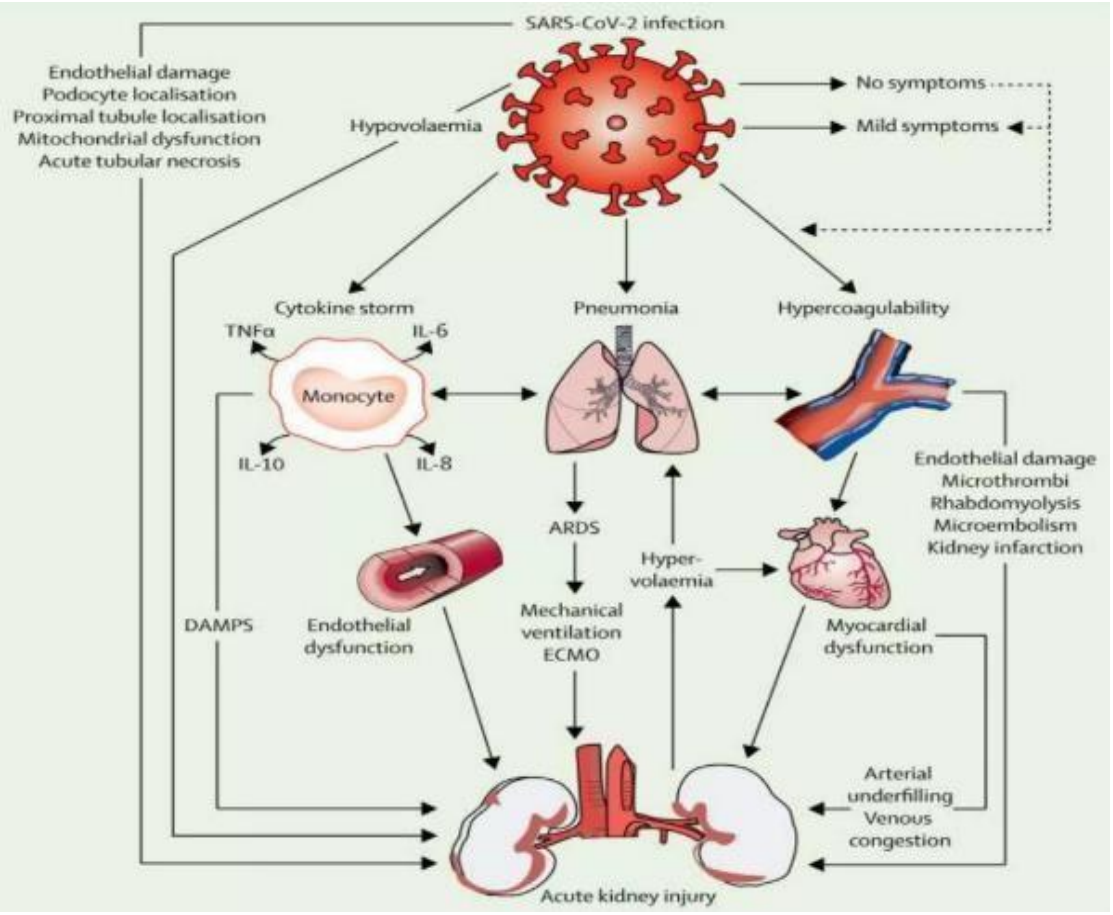
impact) plays an essential role in the consequences of COVID-19. The greatest viral loads were seen in the initial stages in COVID-19 patients, according to other studies.

SARS-CoV-2 R.N.A. Detection-

Following the manufacturer's protocol, RNA was extracted from the samples. Materials required are- Calibrated micropipettes, vortex mixer, Thermoshaker, Microcentrifuge with the rotor, Cooling block, Centrifuge tubes, Sterile gloves, facemask, head cap and lab coats. A Maverick nucleic acid extraction kit was used for extraction of RNA. The total RNA was extracted from a 200-microlitre sample, and a polymerase chain reaction was performed. Following are the steps in RNA isolation using Maverick nucleic acid extraction kit: Sample lysis, Binding and washing, and Elution. RNA (0.5-1 μLg) was added to the final PCR reaction. A quantitative RTPCR targeting the SARS-CoV-2 E gene was used to detect the genome equivalents of SARS-CoV-2. RTPCR analysis, as well as data processing, were conducted with the Real-Time PCR system[22,25,27]. The procedure has been done in ESCO's Biosafety cabinet class II A2 instrument.







A few similar studies have studied urine biochemical parameters and the status of the SARS-COV-2 virus in the urine.

Liu R, Ma Q et al., in 2019, studied **119 cases**, along with 45 healthy controls and their study results described the clinical significance of urine analysis for predicting the severity of coronavirus disease[1].

Ren J .et al. 2019 found that a urine nucleic acid test came positive in an asymptomatic patient. [23]

Peng et al. in 2020 showed that out of 9 patients, one showed the presence of the COVID-19 virus in urine[2].

Kashi AH.et al. found that COVID-19 detection in urine was 3.7% and noted that urine viral load was low in most reports. There was no correlation between the presence of the virus in urine and disease progression[3.]

Ling and colleagues studied 66 patients, of which only four (6.9%) of the 58 patients with viral nucleic acid were found with viral RNA in urine specimens after negative throat swabs. Four patients showed COVID-19 positivity and three had viruses in their urine even after clearance of the virus in oropharyngeal samples[6].

Jin Y, Yang H, Ji W, et al. stated that according to the homogeneity of the viral SARS-COV-2 genome with the SARS virus and the substantial prior

evidence of the presence of the SARS virus in the urine, it was found that the hypothesis of virus transmission by urine originated.[7]

Kim JM .et al. 2020 studied 74 COVID-19 patients and found viral detection rates in all samples and found that the viral transmission rate is low in urine and stool samples. The prevalence of the virus in the urine was 0.8%[22].

Yang Pan. et al. 2020 found that viral RNA is not detected in two patients in urine or stool samples. Viral loads in sputum samples were greater than in throat swab samples[26].

NORMAL VALUES OF URINE PARAMETERS[35]:

Characteristic	Normal Value
Color	Yellow
Clarity	Clear
Specific gravity	1.003-1.030
pH	4.5-7.8
Protein	Negative
Bilirubin	Negative
Urobilinogen	Normal in small amounts
Glucose	Negative
Ketones	Negative
Occult blood	Negative

URINE TESTING AND COLLECTION METHODS:

Sample type	Sampling	Purpose
Random specimen	No specific time most common, taken anytime of day	Routine screening, chemical & FEME
Morning sample	First urine in the morning, most concentrated	Pregnancy test, microscopic test
Clean catch midstream	Discard first few ml, collect the rest	Culture
24 hours	All the urine passed during the day and night and next day Ist sample is collected.	used for quantitative and qualitative analysis of substances
Postprandial	2 hours after meal	Determine glucose in diabetic monitoring
Supra-pubic aspired	Needle aspiration	Obtaining sterile urine

Within two hours of collection, urine samples must be tested in a laboratory to ensure accurate results.

MATERIALS AND METHODS

The study population consisted of patients admitted to the COVID ward in "BLDE(Deemed to be University) Shri B.M.Patil Medical College, Hospital and Research Centre, Vijayapura."

STUDY PERIOD: December 1st 2020, to May 30th 2022.

INCLUSION CRITERIA:

All RT-PCR-positive COVID-19 patients admitted in "BLDE(Deemed to be University) Shri B.M.Patil Medical College, Hospital and Research Centre, Vijayapura" were included.

EXCLUSION CRITERIA:

RT-PCR-positive COVID-19 patients associated with Hypertension, Diabetes and other underlying renal conditions of all age groups were excluded.

SAMPLE SIZE:

With the Anticipated Proportion of Urine infection among COVID-19 patients at 3.7%[3], the sample size for the study was taken as 50 with 95% confidence level and 5% absolute precision.

Sample size calculation-

Total sample size

Formula used is

$n=z^2p*q/d^2$

Where Z= Z statistic at α level of significance

d^2 = Absolute error

P= Proportion rate

q= 100-p

Statistical Analysis:

Results will be presented as Mean+/-SD, counts, percentages and diagrams. Association between variables will be assessed with the help of the Chi-square test.

STATISTICAL ANALYSIS

The obtained data were entered into a Microsoft Excel sheet, and statistical analysis was performed using a statistical package for the social sciences (Version 17). Results are presented as drawings, Mean ±standard deviation (S.D.), counts, and percentages. Results were compared using an independent t-test, and Chi-

square test, and the correlation between variables will be found using the correlation coefficient. All characteristics will be summarized descriptively. The summary statistics of N, mean, and standard deviation(SD) will be used for common variables. The number and percentage will be used in the data summaries for categorical data. Data will be analysed by Chi-square test for association, comparison of means using t-test, ANOVA and ROC analysis.

SAMPLE COLLECTION:

After informed consent, urine samples under aseptic precautions were collected in a sterile container to analyze urine biochemical parameters.

METHODS OF COLLECTION OF DATA:

The study included a total of 50 patients who were admitted to the COVID ward. 10-20 ml of clean midstream urine samples were obtained from the patients. All the collected specimens were tested within 2 hours and to confirm the presence of viral RNA in the urine of COVID -19 positive patients, RT-PCR was done.

CT severity scores of all patients were recorded to assess the disease severity (mild: <9, moderate: 9-15 and severe: >15). The HRCT scores in patients tested for urine RTPCR were analyzed, and the correlation of biochemical parameters with severity was assessed with the help of CT severity scores.

The cycle threshold(Ct) values of all urine RTPCR-positive patients were also recorded.

PARAMETERS IN URINE	RESULTS
PROTEIN	
GLUCOSE	
BLOOD	
BILIRUBIN	
KETONES	
URINE RT-PCR POSITIVITY	

URINE PROCESSING METHOD

10-20 ml of clean midstream urine samples were obtained from the patients

All the collected specimens were tested within 2 hours.

All the urine samples were run in the LAURA SMART urine analyser for chemical parameters and the results were recorded accordingly.

For viral detection in urine, RT-PCR was done.

Urine parameters measured: Protein, Glucose, Blood, specific gravity, bilirubin, Ketone, and Potential of hydrogen(pH).

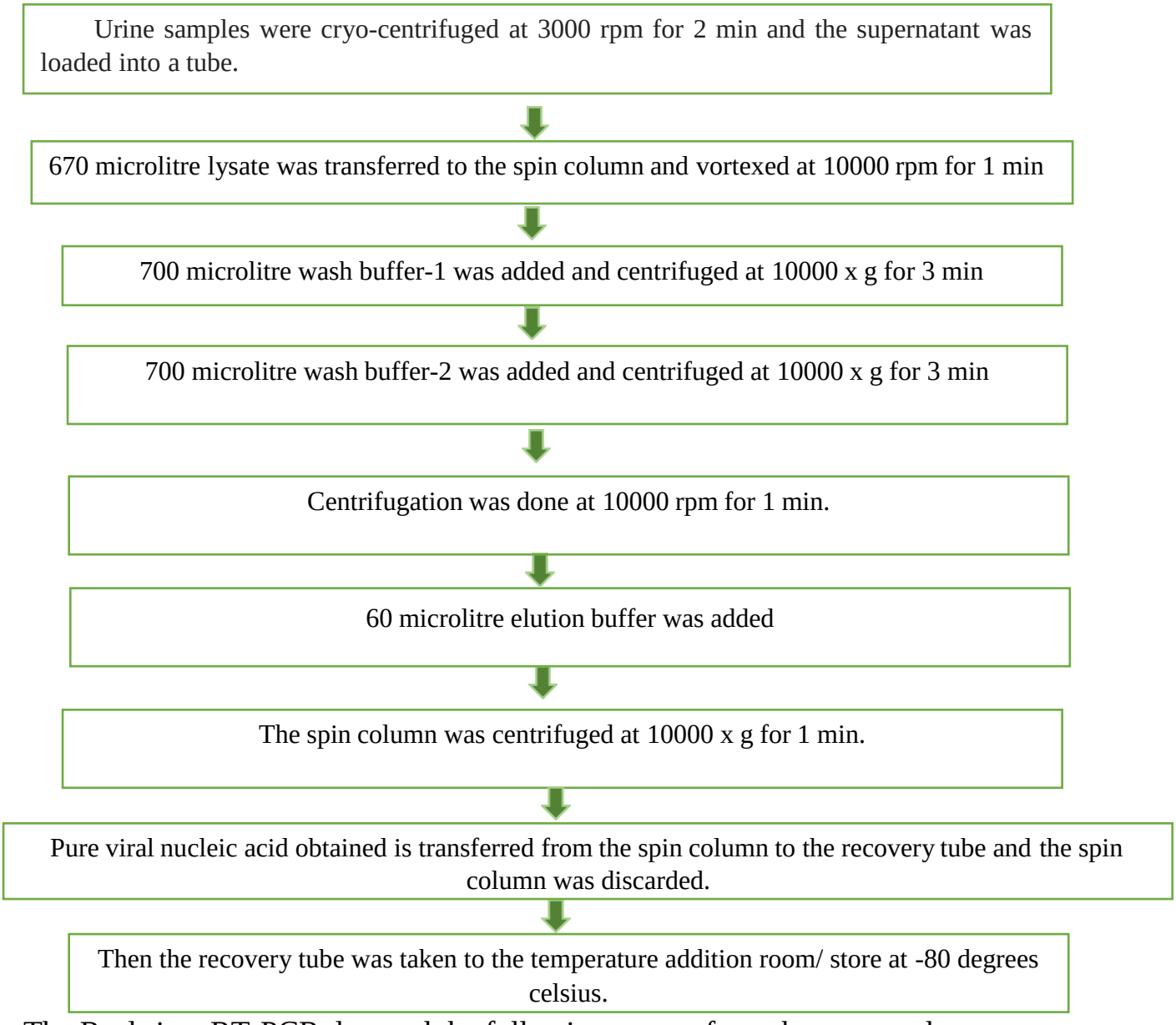
Mylab Discovery solutions- Maverick nucleic acid extraction kit has been used for the study of RT-PCR test in the urine.

LAURA SMART urine analyser

PROCEDURE FOR THE RTPCR TEST IN THE URINE:

MANUAL RNA ISOLATION USING MY LAB

Urine samples were stored in a refrigerator before nucleic acid extraction and RT-PCR.



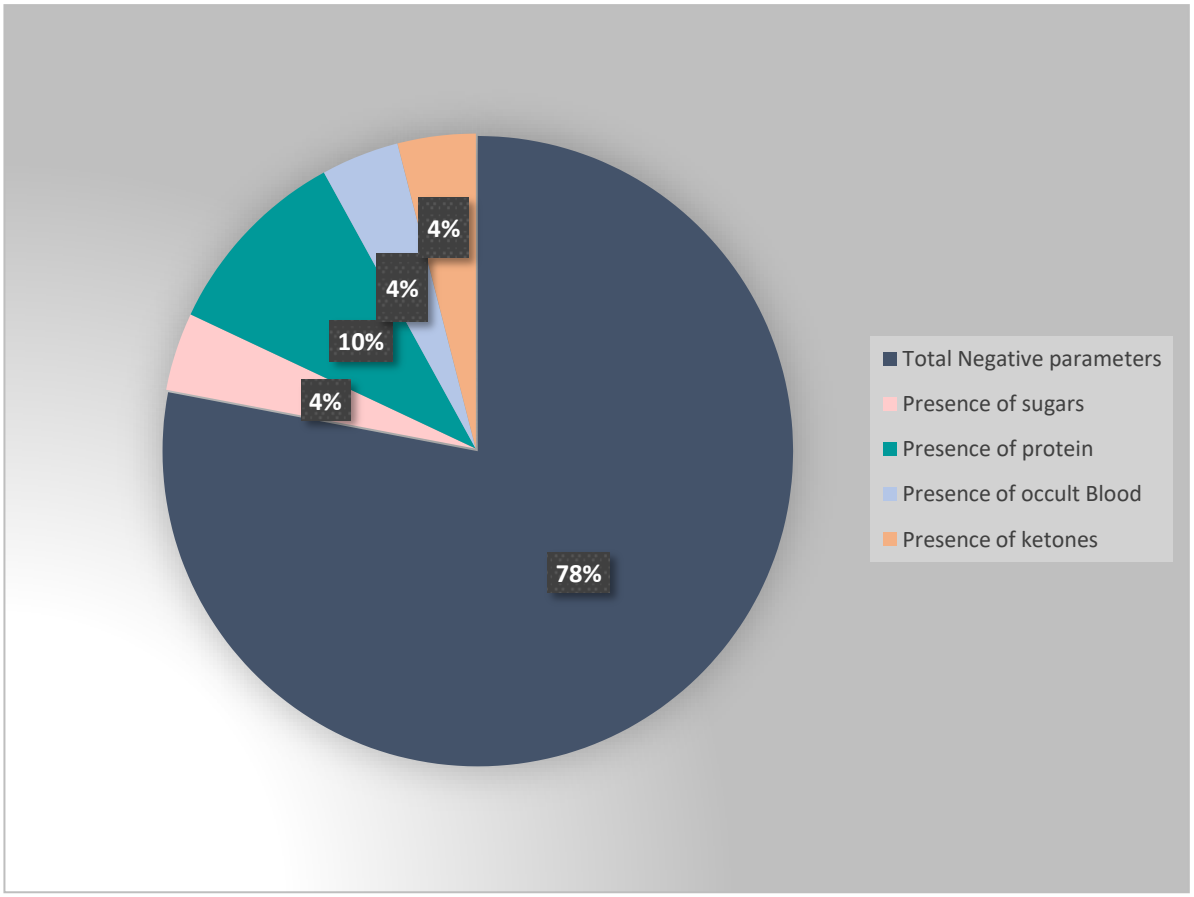
The Real-time RT-PCR detected the following targets from the extracted nucleic acid: RdRP gene and N gene specific for SARS-CoV-2 and E gene in a single tube. A cycle threshold value (Ct-value) less than 39 was defined as a positive test, and a Ct-value of 40 or more was defined as a negative test [32].

RESULTS

Our study was done at the Department of Pathology, "BLDE(Deemed to be University) Shri B.M.Patil Medical College, Hospital and Research Centre, Vijayapura". In our study, we studied 50 patients admitted to the COVID wards. Urine samples were obtained from all the patients, and within 2 hours, all the collected samples were tested. The samples were refrigerated if the testing got delayed.

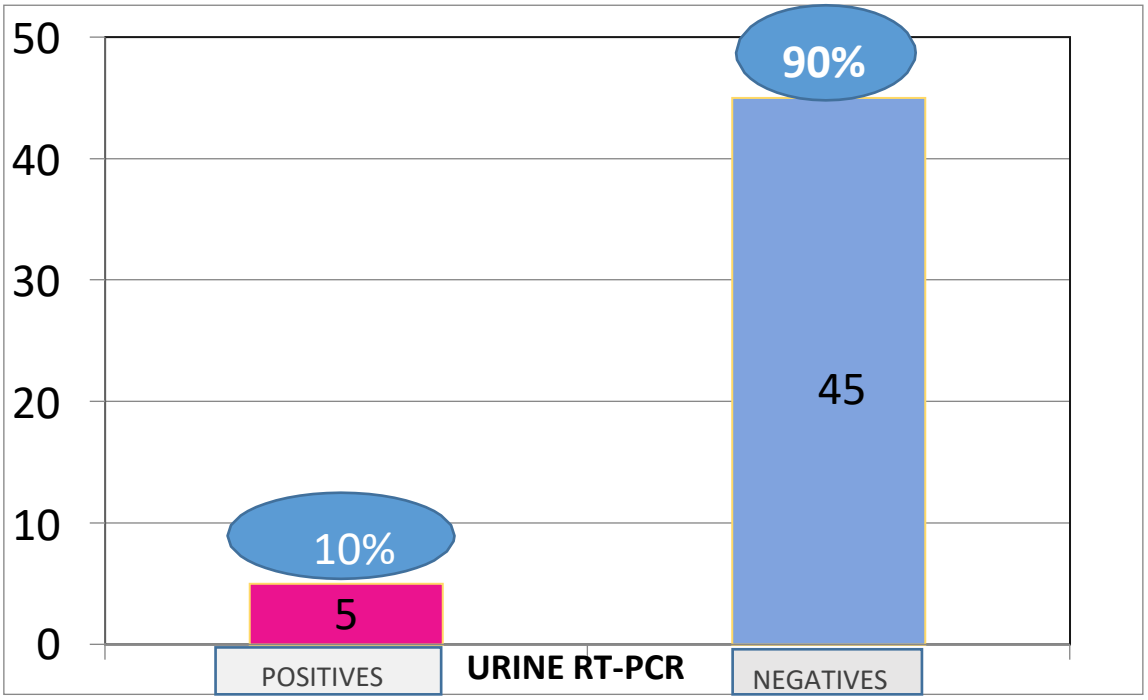
A total of 50 cases were studied. Urine biochemical parameters such as proteins, glucose, blood, bilirubin, ketones, and pH were analyzed and values were documented; even RTPCR for all the urine samples were tested and results were documented.

Here, we present an evaluation of the results of our study.



PERCENTAGE DISTRIBUTION OF PATIENTS OF PATIENTS SHOWING THE URINE CHEMICAL PARAMETERS IN CORONAVIRUS DISEASE 2019 PATIENTS

Out of the total cases, two showed abnormal sugar, five showed abnormal protein, two showed abnormal occult blood and two showed abnormal ketones accounting for 4%, 10%, 4% and 4%, respectively.



BAR DIAGRAM SHOWING URINE RT-PCR RESULTS IN COVID-19-POSITIVE PATIENTS

Five out of 50 patients showed positivity for urine RT-PCR, indicating that the rate of COVID-19 in urine was 10%.

AGE DISTRIBUTION

Among all the study's patients (N = 50), the majority were in the age group ranging from 46-60 years, comprising 16 cases (32% of the study population). The detailed representation is shown below.

AGE (YEARS)	NO. OF PATIENTS	PERCENTAGE (%)
<14	1	2%
15 - 30	7	14%
31 - 45	15	30%
46 - 60	16	32%
61 - 75	7	14%
76-85	4	8%
TOTAL	**50**	**100%**

DISTRIBUTION OF PATIENTS ACCORDING TO THE AGE

GENDER DISTRIBUTION

Among all the patients included in this study were 42 males and eight females, comprising 16% and 84% of total cases, respectively.

SEX	NUMBER	PERCENTAGE (%)
FEMALE	8	16
MALE	42	84
TOTAL	**50**	**100**

GENDER DISTRIBUTION OF ALL THE PATIENTS AND THE NUMBER OF PATIENTS IN EACH GROUP WITH PERCENTAGE

	SAMPLES	HRCT SCORE		
		Mild	Moderate	Severe
TOTAL	50	9	22	19
RTPCR +VE IN URINE	05	01	02	02
RTPCR -VE IN URINE	45	8	20	17

URINE RTPCR WITH GRADING OF HRCT SCORES

C.T. SEVERITY SCORE	NUMBER OF PATIENTS TESTED (SWAB RT-PCR)	NUMBER OF PATIENTS WITH URINE RT-PCR STATUS	CYCLE THRESHOLD (ct)
<9(MILD)	9	01	E and Rdrp gene: cycle threshold value of 33,34
9-15(MODERATE)	22	02	E and Rdrp gene: cycle threshold value of 30 and 31 and 30
>15(SEVERE)	19	02	E and Rdrp gene: cycle threshold value of 30 and 29

RTPCR POSITIVITY WITH C.T. SEVERITY SCORES AND CYCLE THRESHOLD VALUES IN URINE RTPCR-POSITIVE PATIENTS

RT-PCR methods give an estimate of viral load. The PCR reaction cycle threshold values are inversely proportional to the viral load; low ct values indicate high viral loads and disease severity and vice versa.

Urine RTPCR	**PRESENCE OF PROTEIN**	**ABSENCE OF PROTEIN**	**p-value**
POSITIVE(5)	One patient(++) Three patients: (traces of albumin)	One patient	**<0.001***
NEGATIVES(45)	One patient(1+)	44 patients	

Table 06- Estimation of protein in urine in all the patients

Note: p-value* significant at 5% level of significance ($p<0.05$)

Among all the patients included in this study, five patients showed the presence of protein in the urine, whereas 45 patients showed the absence of protein in the urine, comprising 10 % and 90 % of total cases, respectively.

Urine RTPCR	**PRESENCE OF SUGAR**	**ABSENCE OF SUGAR**	**p-value**
POSITIVE(5)	One patient: One(++)	Four patients	**0.0007***
NEGATIVES(45)	One patient: One (1+)	44 patients	

Table 07- Estimation of sugar in urine in all the patients

Among all the patients included in this study, two patients showed the presence of sugar in the urine, whereas 48 patients showed the absence of sugar in the urine, comprising 4% and 96% of total cases, respectively.

Total =50 (100%)	**Presence of Occult blood**	**Presence of Ketones**	**Presence of pH/ Urobilinogen**
5(10%)- Urine RT-PCR POSITIVE	One patient: (50 RBCs/microlitre)	One patient: One (Trace)	Nil
45(90%)- Urine RT-PCR NEGATIVE	One patient: (10 RBCs/microlitre)	One patient: One (1+)	Nil

Table 07- Estimation of blood and ketones and others in urine in all the patients

Out of all patients, two patients showed blood in the urine, and two patients showed ketones in the urine. Rest all other parameters are normal in all COVID-19-positive patients.

DISCUSSION

Severe acute respiratory syndrome- corona virus-2 (SARS- COV-2) binds to the angiotensin-converting enzyme 2 (ACE-2) receptors and thereby plays a role in cellular2 entry to cause Infection. Furthermore, A.C.E.2 upregulated is also seen in the heart, renal system and genital organs. Therefore multiple organ involvement, including the kidney, by SARS-COV-2 Infection has been reported.

The following two mechanisms have been proposed for virus transmission through urine:

One theory is that sepsis and cytokine storm affects the renal system and causes viral shedding into the urine.

Another theory is that the virus binds to ACE 2 receptors involving the urinary tract resulting in viral shedding into the urine.

As a result of general population susceptibility, SARS-CoV-2 has spread rapidly globally despite its reduced pathogenicity.

The Clinical symptoms in COVID-19-infected individuals are breathlessness, cough, and fatigue[19]. Patients with underlying diseases and the elderly experience severe infection and can cause sepsis, multiple organ damage, and death.

The swab RTPCR has been the preliminary investigation for the diagnosis of COVID-19. The RT-PCR for SARS-CoV-2 can be performed on faeces, stool and urine, although throat swabs are most common. For instance, where a sample

from the lower respiratory tract may be positive for RTPCR, a throat swab may not. Therefore, if the screening test on throat swabs was negative, an RTPCR in urine can be used as a complimentary test.

Coronavirus disease 2019 is spread mainly by direct contact and inhalation of respiratory droplets. There is no proof that the virus may be transmitted orally through faeces, despite being isolated from faeces. It has been shown from prior research that this is how MERS-CoV is spread[20]. The results showed that few patients were urine RT-PCR positivity, suggesting that the patient could be a source of Infection and might excrete the virus. Nevertheless, we were unable to isolate a virus from our patient's urine or send samples to another lab that would pose a risk of transmission.

Faeces and urine collection are more convenient, and it is simple to determine the number and quality of specimens compared to nasopharyngeal swabs and sputum samples. These samples could increase the detection of asymptomatic patients even. A major problem in preventing the pandemic might arise if patients can spread the virus through droplets and other sources. Early diagnosis is the mainstay for improving patient outcomes and has significantly contributed to boosting the research for innovative biomarkers. On-time diagnosis and apt management of COVID patients is a challenge, especially when there is multiple organ involvement.

There have been few studies on the association between COVID-19 and urine biochemical markers. Following are a few similar studies whose results are in concordance with our study.

In our study, urinary biochemical parameters were also analyzed, and it was found that all five urine RT-PCR-positive patients showed abnormal urine biochemical parameters.

Our study results are in concordance with the study done by **Liu R, Ma Q et al.** in 2019. They studied **119 cases** and 45 healthy controls and their study results described the clinical significance of urine analysis for predicting the severity of coronavirus disease[1]. Out of 119 cases, a higher rate of urine glucose positivity and PRO was observed in the severe groups. Urine occult blood and proteinuria in COVID-19 patients are more than in healthy controls. In our study, We found an increase in proteinuria and glucosuria in patients with positive urine RT-PCR results. Seven of these patients showed severe HRCT scores, implying a correlation between biochemical parameters with their severity.

Bonetti G et al. in 2019 found that out of 226 COVID-19 patients, proteinuria(203/226) and hematuria(163/226) were found in many patients[4]. **In our study**, proteinuria is seen in more patients.

Ren J .et.al. 2019 found that a urine nucleic acid test came positive in an asymptomatic patient.[23]

Our study results are in concordance with the study done by **Peng et al**. in 2020 showed that out of 9 patients, one showed the presence of the COVID-19 virus in urine[2]. **In our study**, 5 cases out of 50 were found positive for urine RT-PCR.

Kashi AH.et al. found that COVID-19 detection in urine was 3.7% and noted that urine viral load was low in most reports. There was no correlation between the presence of the virus in urine and disease progression[3]. **In our study,** it was found that the rate of COVID-19 presence in urine was 10%.

Ling and colleagues studied 66 patients; only four (6.9%) of the 58 patients with viral nucleic acid were found with viral RNA in urine specimens after negative throat swabs[6]. **In this study,** five cases(10%) out of 50 were positive for urine RT-PCR.

Jin Y, Yang H, Ji W, et al. found the idea of virus transmission through urine originated from the homogeneity of the viral SARS-COV-2 genome with the SARS virus and the abundant previous evidence of the presence of the SARS virus in the urine.[7]

Kim JM .et.al. 2020 studied viral detection rates in all samples collected from 74 COVID-19 patients and found that the viral transmission rate is low in urine samples. The virus detection rate in the urine samples was 0.8%.[22]

Yang Pan. et al. 2020 found no viral RNA was detected in urine or stool samples from two patients[26].

Erdogan O et al. 2021 found that out of 133 COVID-19 patients, erythrocyturia, proteinuria, and glucosuria rates were significantly higher in patients than in the controls[28].

PARAMETERS	STUDY	RESULTS
URINE BIOCHEMICAL PARAMETERS	**Liu R, Ma Q et al. in 2019**	Out of 119 COVID-19 patients, a higher rate of urine glucose positivity and protein rate was observed in the critical and severe groups than in the moderate groups. Urine occult blood and proteinuria in COVID-19 patients is more than in healthy controls[1].
	Bonetti G et al. in 2019	Out of 226 COVID-19 patients, proteinuria(203/226) and hematuria(163/226) found in many patients[4].
	Erdogan O et al. in 2021	Out of 133 COVID-19 patients, erythrocyturia, proteinuria and glucosuria rates were significantly higher in patients than in the controls[28].
	In present study	Out of 50 COVID-19-positive patients, increase in proteinuria and glucosuria in patients with positive urine RT-PCR results. Seven of these patients showed severe HRCT scores, implying a correlation between biochemical parameters with their severity.

PARAMETERS	STUDY	RESULTS
URINE RTPCR POSITIVITY	**Ren J.et al. 2019**	Found that in an asymptomatic patient, urine nucleic acid test came positive[23].
	Peng. et al. in 2020	Out of 9 patients, one showed the presence of the COVID-19 virus in urine[2].
	Kashi AH.et.al.	Found the rate of COVID-19 presence in the urine sample was 3.7%[3].
	Ling and colleagues	Only four (6.9%) of the 58 patients with viral nucleic acid were found with viral RNA in urine specimens after negative throat swabs[6].
	Kim JM .et al. 2020	Viral detection rates in all samples collected from 74 COVID-19 patients and found that the viral transmission rate is low in urine samples. The virus detection rate in the urine was 0.8%[22]
	Yang Pan. et al. 2020	No viral RNA was detected in urine or stool samples from two patients[26].
	In present study	Five cases(10%) out of 50 were positive for urine RT-PCR, and one patient died of more severe disease.

SUMMARY

This study was done at the Department of Pathology, B.L.D.E. (Deemed to be University), Shri B. M. Patil Medical College, Hospital and Research Centre, Vijayapura, Karnataka.

In our study, we studied 50 patients admitted to the COVID ward and fulfilled the inclusion and exclusion criteria.

We studied urine biochemical parameters in the LAURA SMART urine analyzer and viral RT-PCR testing with the help of the MAVERICK NUCLEIC ACID EXTRACTION KIT. Proteins, glucose, blood, leukocytes, bilirubin, ketones, and pH were all analyzed in the study.

Salient observations from our study are,

Urinary biochemical parameters were analysed, and out of 50 COVID-19-positive patients, 2 showed abnormal sugar(one patient 1+; other patient ++), 5 showed abnormal protein(traces of albumin), and 2 showed abnormal occult blood and 2 showed abnormal ketones.

Five cases showed positivity for urine RT-PCR with a cycle threshold(Ct) value of around 35, and one patient died as a result of a more severe disease, as evidenced by follow-up studies on these patients.

Out of 5 patients who showed urine RT-PCR positivity, one patient showed abnormal protein(++) and three showed traces of albumin, and one showed abnormal occult blood. One patient showed abnormal ketones when urine

biochemical parameters were analysed. The urine RT-PCR-positive patients showed higher levels of abnormal urine biochemical parameters than those who did not have RT-PCR results.

CONCLUSION

COVID-19 patients manifests with proteinuria, glucosuria, haematuria and ketonuria. These patients might signify its impact on the kidney and severe COVID-19 Infection can lead to severe kidney effects and subsequent renal failure. Furthermore, the SARS-COV-2 virus in urine indicates that covid-19 patients can shed the virus in the urine. So, clinicians and pathologists should be aware of these consequences for successfully further disease management and also be aware that transmission of the disease through urine is possible.

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ANNEXURE – I

B.L.D.E. (DEEMED TO BE UNIVERSITY) IEC/no-09/2021 Date-22/01/202

(Declared vide notification No. F.9-37/2007-U.3 (A) Dated. 29-2-2008 of the MHRD, Government of India under Section 3 of the UGC Act, 1956)

The Constituent College

SHRI. B. M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Institutional ethical committee of this college met on 11-01-2021 at 11-00 am to scrutinize the synopsis of Postgraduate students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected and revised version synopsis of the Thesis has been accorded Ethical Clearance

Title: Study of urine parameters and presence of virus in covid patients.

Name of PG student: Dr Karukola Pratyusha, Department of Pathology

Name of Guide/Co-investigator: Dr S.B.Hippargi , Professor of Pathology

DR.S.V.PATIL
CHAIRMAN, IEC
Institutional Ethical Committee
B L D E (Deemed to be University)
Shri B.M. Patil Medical College,
VIJAYAPUR-586103 (Karnataka)

Following documents were placed before Ethical Committee for Scrutinization:

1. Copy of Synopsis / Research project
2. Copy of informed consent form
3. Any other relevant documents.

ANNEXURE-II

B.L.D.E (DEEMED TO BE) UNIVERSITY,

SHRI B.M.PATIL MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTER, VIJAYAPURAA-586103

INFORMED CONSENT FOR PARTICIPATION IN DISSERTATION/RESEARCH

I, the undersigned, , S/O D/O W/O , aged years, ordinarily resident of _ do hereby state/declare that Dr of Hospital has examined me thoroughly on at (place) and it has been explained to me in my own language that I am suffering from disease (condition) and this disease/condition mimic following diseases. Further Doctor informed me that he/she is conducting dissertation/research titled under the guidance of Dr requesting my participation in the study. Apart from routine treatment procedure, the pre-operative, operative, post-operative and follow-up observations will be utilized for the study as reference data.

Doctor has also informed me that during conduct of this procedure adverse results may be encountered. Among the above complications most of them are treatable but are not anticipated hence there is chance of aggravation of my condition and in rare circumstances it may prove fatal in spite of anticipated diagnosis and best treatment made available. Further Doctor has informed me that my participation in this study will help in evaluation of the results of the study which is useful reference to treatment of other similar cases in near future, and also I may be benefited in getting relieved of suffering or cure of the disease I am suffering.

The Doctor has also informed me that information given by me, observations made/ photographs/ video graphs taken upon me by the investigator will be kept secret and not assessed by the person other than me or my legal hirer except for academic purposes.

The Doctor did inform me that though my participation is purely voluntary, based on information given by me, I can ask any clarification during the course of treatment / study

related to diagnosis, procedure of treatment, result of treatment or prognosis. At the same time I have been informed that I can withdraw from my participation in this study at any time if I want or the investigator can terminate me from the study at any time from the study but not the procedure of treatment and follow-up unless I request to be discharged.

After understanding the nature of dissertation or research, diagnosis made, mode of treatment, I the undersigned Shri/Smt under my full conscious state of mind agree to participate in the said research/dissertation.

Signature of patient:

Signature of doctor: Witness:

1.

2.

Date:

Place:

ANNEXURE - III

PROFORMA

NAME : OP/IP No.:

AGE :

SEX : Date of Admission:

RELIGION : Date of Discharge:

OCCUPATION :

RESIDENCE :

PRESENTING COMPLAINTS :

PAST HISTORY :

PERSONAL HISTORY :

FAMILY HISTORY :

TREATMENT HISTORY :

GENERAL PHYSICAL EXAMINATION**:**

Pallor	Present/Absent
Icterus	Present/Absent
Clubbing	Present/Absent
Lymphadenopathy	Present/Absent
Edema	Present/Absent
Built	Poor/Average/Well

VITALS: PR: RR:

BP: TEMPERATURE:

ADDITIONAL DATA:

MAP (Mean Arterial Pressure)

Urine Output

GCS (Glasgow Coma Scale)

SOFA (Sequential Organ Failure Assessment) Score

SYSTEMIC EXAMINATION:

Cardiovascular system:

Respiratory system:

Per Abdomen:

Central nervous system:

Clinical Diagnosis:

INVESTIGATIONS:

Red blood cell count (RBC count):

Hemoglobin (Hb):

Total Leucocyte Count (TLC):

Platelet Count (PC):

Immature Platelet Fraction (IPF%):

Reticulocyte count (RET%):

Bilirubin:

Creatinine:

Blood Culture:

Other Investigations:

SL. NO	AGE	SEX	PROTEINS	SUGAR	BLOOD	KETONES	pH	BILIRUBIN/UROBILINOGEN	URINE RT-PCR
1	41	F	Absent	Absent	Negative	Absent	7.3	-ve/ 0.1mg/dl	Negative
2	30	M	Absent	Absent	Negative	Absent	6.5	-ve/ 0.1mg/dl	Negative
3	65	M	Absent	Absent	Negative	Absent	5.5	-ve/ 0.1mg/dl	Negative
4	52	M	Absent	Absent	Negative	Absent	6.3	-ve/ 0.1mg/dl	Negative
5	45	M	Absent	Absent	Negative	Absent	6.3	-ve/ 0.1mg/dl	Negative
6	52	M	Absent	Absent	Negative	Absent	6.2	-ve/ 0.1mg/dl	Negative
7	38	M	Absent	Absent	Negative	Absent	6	-ve/ 0.1mg/dl	Negative
8	52	M	Absent	Absent	Negative	Absent	7.5	-ve/ 0.1mg/dl	Negative
9	53	M	Absent	Absent	Negative	Absent	7	-ve/ 0.1mg/dl	Negative
10	54	M	1+(30 mg/dl)	Absent	Negative	Absent	6.8	-ve/ 0.1mg/dl	Negative
11	27	M	Absent	Absent	10 ery/microL	Absent	7	-ve/ 0.1mg/dl	Negative
12	63	M	Absent	Absent	Negative	Absent	7.2	-ve/ 0.1mg/dl	Negative
13	40	M	Absent	1+ (50 mg/dl)	Negative	Absent	7	-ve/ 0.1mg/dl	Negative
14	45	M	Absent	Absent	Negative	Absent	6.02	-ve/ 0.1mg/dl	Negative
15	59	M	Absent	Absent	Negative	Absent	6	-ve/ 0.1mg/dl	Negative

16	55	M	Absent	Absent	Negative	Absent	6.2	-ve/ 0.1mg/dl	Negative
17	75	F	++(100 mg/dl)	Absent	++ (50 ery/microL)	Absent	5.8	-ve/ 0.1mg/dl	Positive
18	81	M	Absent	Absent	Negative	Absent	6.3	-ve/ 0.1mg/dl	Negative
19	40	M	Absent	Absent	Negative	Absent	6.2	-ve/ 0.1mg/dl	Negative
20	60	M	Absent	Absent	Negative	Absent	6	-ve/ 0.1mg/dl	Negative
21	42	M	Absent	Absent	Negative	Absent	6	-ve/ 0.1mg/dl	Negative
22	23	M	Absent	Absent	Negative	Absent	7.2	-ve/ 0.1mg/dl	Negative
23	53	M	Absent	Absent	Negative	Absent	5.5	-ve/ 0.1mg/dl	Negative
24	35	M	Absent	Absent	Absent	Absent	6.2	-ve/ 0.1mg/dl	Negative
25	36	M	Absent	Absent	Negative	Absent	7	-ve/ 0.1mg/dl	Negative
26	29	M	Absent	Absent	Negative	Absent	7	-ve/ 0.1mg/dl	Negative
27	35	M	Absent	Absent	Negative	1+ (16mg/dl)	6	-ve/ 0.1mg/dl	Negative
28	60	F	Absent	Absent	Negative	Absent	5.5	-ve/ 0.1mg/dl	Negative
29	29	M	Absent	Absent	Negative	Absent	5	-ve/ 0.1mg/dl	Negative
30	65	M	Absent	Absent	Negative	Absent	5	-ve/ 0.1mg/dl	Negative
31	54	M	Absent	Absent	Negative	Absent	6.2	-ve/ 0.1mg/dl	Negative
32	79	M	Absent	Absent	Negative	Absent	6	-ve/ 0.1mg/dl	Negative

33	40	M	Absent	++(100 mg/dl)	Absent	Absent	6.2	-ve/ 0.1mg/dl	Positive
34	45	M	Absent	Absent	Negative	Absent	5	-ve/ 0.1mg/dl	Negative
35	78	F	Absent	Absent	Negative	Absent	5	-ve/ 0.1mg/dl	Negative
36	29	M	Absent	Absent	Negative	Absent	5.5	-ve/ 0.1mg/dl	Negative
37	27	M	Absent	Absent	Negative	Absent	5.5	-ve/ 0.1mg/dl	Negative
38	13	M	Traces	Absent	Negative	Absent	6.5	-ve/ 0.1mg/dl	Positive
39	60	F	Absent	Absent	Negative	Absent	5	-ve/ 0.1mg/dl	Negative
40	70	M	Absent	Absent	Negative	Absent	6.5	-ve/ 0.1mg/dl	Positive
41	34	M	Absent	Absent	Negative	Absent	5	-ve/ 0.1mg/dl	Negative
42	70	M	Traces	Absent	Negative	Trace (5.2 mg/dl)	6	-ve/ 0.1mg/dl	Positive
43	53	F	Absent	Absent	Negative	Absent	7	-ve/ 0.1mg/dl	Negative
44	79	M	Absent	Absent	Negative	Absent	6	-ve/ 0.1mg/dl	Negative
45	31	F	Absent	Absent	Negative	Absent	6	-ve/ 0.1mg/dl	Negative
46	60	F	Traces	Absent	Absent	Absent	6.5	-ve/ 0.1mg/dl	Positive
47	54	M	Absent	Absent	Negative	Absent	6.5	-ve/ 0.1mg/dl	Negative
48	38	F	Absent	Absent	Negative	Absent	6.5	-ve/ 0.1mg/dl	Negative
49	67	M	Negative	Negative	Negative	Absent	6.5	-ve/ 0.1mg/dl	Negative
50	41	F	Absent	Absent	Negative	Absent	6.5	-ve/	Negative

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