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KETONURIA AN EARLY COVID-19 SEVERITY & PROGNOSTIC MARKER? AN OBSERVATIONAL STUDY.

Medical Science

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ABSTRACT

Ketone bodies are insignificant in the blood and urine of normal individuals. Ketoacids become important sources of metabolic energy in circumstances in which the availability of glucose is restricted, as during prolonged fasting, or when the ability to use glucose is greatly diminished, as in decompensated diabetes mellitus or any infective illness like viral or bacterial infection1.It has been found that increased utilization of glucose for CORONA virus replication. Codo et al.1 provided conclusive evidences that glycolytic flux is indispensable for SARS-CoV-2's impact. Increased utilization of glucose for CORONA virus replication and flux results in diminished availability of glucose and therefore increased production of ketone bodies and its appearance in urine. This study assesses the level of ketonuria and disease severity and use of ketonuria as prognostic marker of COVID infection.

KEYWORDS

COVID, Ketonuria, SARS-CoV-2, AMP-kinase, Reactive oxygen species, CORONA, Aerobic, Glycolysis, β-Oxidation, Fatty Acid, Ketone bodies.

INTRODUCTION

Viruses alter the host cell metabolism in order to make optimal conditions for their rapid and efficient replication and spread. Enhanced uptake of important nutrients such as glucose to support metabolic signaling, i.e., aerobic glycolysis, a primary pathway of glucose metabolism and its by-products for biosynthetic reactions. The increased glucose metabolism imposed by sustained hyperglycemia may enhance SARS-CoV-2's entry and subsequent replication as well as an exacerbated immune response in individuals with diabetes[2].

Codo et al. explored the molecular response of SARS-CoV-2 infected human monocytes under diabetic condition. The authors initially show that dose-dependent increase in glucose concentrations potentiate SARS-CoV-2 replication as well as ACE2 upregulation and cytokine production in monocytes suggesting elevated glucose as a principal promoter of virus replication and inflammatory response[1].

SARS-CoV-2 directly induces glycolysis in monocytes, which is in line with the enrichment of glycolytic genes and metabolic remodeling observed by single-cell RNA sequencing (RNA-seq) of lung monocyte. Codo et al. provided conclusive evidences that glycolytic flux is indispensable for SARS-CoV-2's impact. Through well-designed experiments, the authors show that inhibition of glycolysis by 2-deoxy-D-glucose (2-DG) as well as inhibition of glycolytic enzymes 6-phospho-fructo-2-kinase/fructose-2,6-bisphosphatase-3 (PFKFB3), a positive regulator of phosphofructokinase-1 (PFK1) as well as lactate dehydrogenase A (LDH-A) abolishes viral replication and cytokine response placing glycolysis as a key upstream event during SARS-CoV-2 pathogenesis[1].

Finally, the authors investigated whether metabolic remodeling and altered immune state of monocytes potentiated by high glucose during SARS-CoV-2 infection impairs T cell function and response. Conditioned media obtained from metabolically primed SARS-CoV-2 infected monocytes compromise CD4 or CD8 T-cell proliferation and also induce lung epithelial cell death[1]. Inhibition of HIF-1α, as well as neutralization of ROS or IL-1β antagonize such effect and restore T-cell function or lung epithelial cell survival, which suggests that increased glucose metabolism through aerobic glycolysis and subsequent cytokine production in monocytes could further deteriorate neighboring cells in a paracrine fashion under pro-diabetic conditions in COVID-19 patients[4].

IL-1β processing is regulated through generation of 3-phosphoglycerate, a by-product of glycolysis suggesting a direct link to glucose metabolism. Furthermore, the inhibition of glycolysis with 2-DG was shown to halt IL-1β induction and to protect against LPS-induced sepsis in mice[4]. Dysregulated glucose metabolism in people with diabetes may explain the increased susceptibility to SARS-CoV-2 and why uncontrolled diabetes can lead to excessive adaptive immune reactions in patients with critical COVID-19 symptoms[3]. This upregulation of glucose production in COVID induces diabetes like state and appearance of ketone bodies in urine.

Since ketone bodies are not bound to plasma proteins, they are freely filterable solutes in the renal glomerulus and appear quantifiably in the tubular urine. At the very low plasma concentrations of ketone bodies that are encountered normally after an overnight fast, urinary excretion rates are negligible. When plasma levels increase beyond 0.1 to 0.2 m*M*, however, excretion increases and measurable amounts of ketone bodies appear in the urine[1,2]. Ketone bodies in urine appears before than ketosis in blood, making it early prognostic marker of severity of infection.

METHODOLOGY

Ketonuria is established and well-versed severity indicator for the Diabetic ketoacidosis. Ketosis is mainly assessed by both serum and urine level. Many causes of ketosis are already established of them in general it is diabetes which is blindly followed in Diabetic ketoacidosis. However, ketosis is present in many infective and metabolic causes where primary utilization of carbohydrate as a fuel is shifted to fatty acid generating ketone bodies. Urine ketonuria appears early than the ketosis in serum. As with growing COVID-19 cases it will be beneficial to develop an early indicator for development of severity which is patho-physiologically indiacted by ketonuria. The aim of this study was to assess the level of ketonuria in diabetic and non-diabetic COVID-19 subjects at the time of admission and compare with COVID related length of stay in the hospital indicating disease burden, severity of infection.

MATERIALS AND METHOD

Patients with COVID-19 who were admitted and tested positive with COVID-19 RT-PCR based test at the institute Microbiology department. Both the patients with and without diabetes were tested for ketonuria.

The sodium nitroprusside reaction measures both acetone and acetoacetate, but not hydroxybutyrate, in biological materials. There is no interference from most drugs and metabolic products. It is the most successful test devised. Nitroprusside is available as a test tablet (Acetest) and as a coated reagent strip (Ketostix), With Ketostix, the strip is momentarily dipped into the urine specimen or passed through

in the urinary stream and compared to a color chart 1 minute later. The scale is negative, trace, small, moderate, and large. The strip is capable of detecting 5 mg/dl acetoacetate but is not reactive to acetone Subjects who were tested positive for COVD-19 by RT-PCR in the institute, a tertiary care hospital were included in the study. No age group or sex segregation was done to remove bias in severity assessment. Both diabetic and non-diabetic subjects were included in the study to prevent diabetes dependent ketoacidosis as a confounding factor. Only COVID infection was considered for assessment. Patients excluded were subjects who are on ventilator bound or with severe acute respiratory syndrome or respiratory disorder cases categorised as serious cases so as to avoid any bias in conversion of subjects from asymptomatic and symptomatic to severe cases. Patients already on Insulin therapy was excluded as Insulin therapy will prevent lipolysis. All patients were assessed for Ketonuria on the day of admission and their numbers of days of stay at hospital and death if any.

Limitation

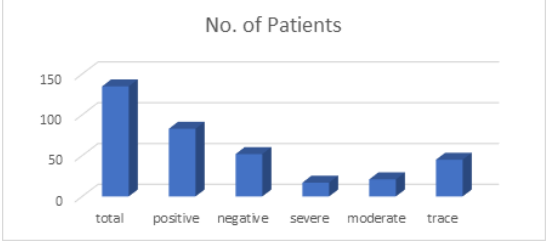
The Ketostix test is most accurate when urines are tested with a high specific gravity (between 1.010 and 1.020) and low-pH. Highly pigmented urine specimens may yield false positive readings. Levodopa will also cause a false positive result. Ketonuria is considered only for infectivity at the day of admission to hospital. No co-relation was done with disease severity by CT-Scan or cytokine storm was done to prevent bias.

RESULT

The study was done using the Ketostix with approximate value to ketone bodies present in the urine as the study was a to assess the level of ketonuria and severity of infection. All subjects with positive COVID RT PCR test were included into the study. Assessment of total number of severe, moderate and mild urine ketonuria was done and burden of disease by longevity of stay in hospital and mortality among the them was done. No age or sex categorisation was done to avoid any bias. Patients who were on insulin therapy were excluded. Total of 135 patients were included in the study between May 2020 till November 2020.

Category	No. of Patients
Total	**135**
Positive	**83**
Negative	**52**
Severe	**17**
Moderate	**21**
Trace	**45**

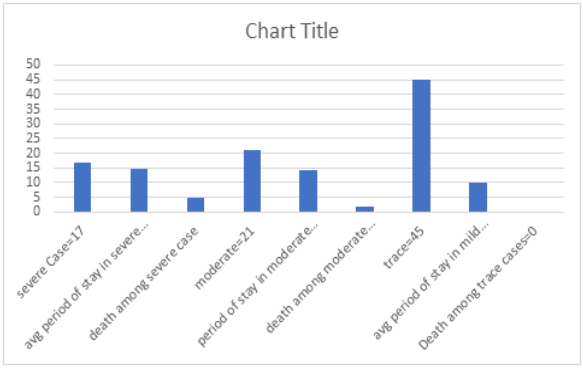
From 135 total number of subjects, 83 (61.5%) subjects were positive for urine ketonuria. 52 (38.5%) found negative. Out of 83 positive subjects, 17 (20.5%) were having Large or severe ketonuria, 21 (25.3%) were having moderate ketonuria, 45 (54.2%) had trace ketonuria.



Severe Case	**17**
Avg period of stay in severe cases	14.6±3
Death among severe case	5
Moderate	**21**
Avg period of stay in moderate cases	14.4±2
Death among moderate cases	2
Trace	**45**
avg period of stay in mild cases=10.1±2	10.1±2
Death among trace cases=0	0

Assessment of burden of disease is done with longevity of stay in hospital due to COVID and associated mortality which indicates ongoing treatment due to COVID. All the subjects were assessed when they were COVID positive and their stay in hospital only. However, burden of disease due to gradual complexities due to COVID was not assessed and is an area of further studies. Limitation of conducting the study in COVID ward is another stopping factor.

On assessment of longevity of stay, average period of stay in hospital in both severe and moderate cases was approximately 14 days and in trace subjects was approximately 10 days only. Death in severe cases was 5 (6.02%) and in moderate cases was 2 (9.5%), in 45 trace cases



On assessment it was found that, from total of 135 subjects included in the study, 17 subjects were having severe ketonuria, 21 had moderate ketonuria, and 45 had trace ketonuria.

Average period of stay among severe and moderate ketonuria was approximately 14 days and among trace ketonuria cases was 10 days. It signifies increased burden of COVID illness due to infection and approximately similar in both severe and moderate cases. Further assessment of correlation by radiological picture like CT Scan and other severity score should be done.

Reference Range of ketone bodies

Urine Value	Designation	Approximate Serum concentration (reference range)	
		Mg/dl	Mmol/l
0	**Negative**	**0.5-3.0**	**0.05-0.29**
TRACE	+	**5**	**0.5**
SMALL	++	**15**	**1.5**
MODERATE	+++	**40**	**4**
LARGE	++++	**80 TO 160**	**8 TO 16**

Information paper SIEMENS Multistix10 SG (Reagent strip for urine analysis)

DISCUSSION

Infections are related to increased production of reactive oxygen species (ROS). These ROS has emerged as the key activator of AMP related protein kinase (AMP-K). The net effect is ATP conservation via on/off switching of metabolic pathways that produce/consume ATP. This can have either pro-survival or pro-apoptotic effects. Study by Donna N Douglas et al demonstrated cytopathic effects of Hepatitis C Virus (HCV)-induced liver injury and are readily observed in Huh7.5 cells infected with the JFH-1 HCV strain. Reactive oxygen species-mediated activation of AMP-activated kinase in Huh7.5 cells during HCV (JFH-1)-induced growth arrest[5,6]. Metabolic labeling experiments provided direct evidence that lipid synthesis is attenuated, and β-oxidation is enhanced in these cells. A striking increase in nuclear peroxisome proliferator-activated receptor α, which plays a dominant role in the expression of β-oxidation genes after ligand-induced activation. The combination of attenuated lipid synthesis and enhanced β-oxidation is not conducive to lipid accumulation, yet cellular lipids still accumulated during this stage of infection. Notably, the serum in the culture media was the only available source for polyunsaturated fatty acids, which were elevated (2-fold) in the infected cells, implicating altered lipid import/export pathways in these cells[5,6]. This study also provided the first *in vivo* evidence for enhanced β-oxidation during HCV infection because HCV-infected SCID/Alb-uPA mice accumulated higher plasma ketones while fasting than did control mice. A similar picture of increased ROS production is seen in COVID and may disarranged β-oxidation giving rise to ketosis and ketonuria.

As seen in this study ketonuria was present in initial presentation of COVID infection with increased infection predictable by more stay in hospital based treatment in moderate to severe cases and observed with higher mortality than trace cases. However, more assessment of early prognostic marker in COVID with severity should be studied.

CONCLUSION

Disturbances in lipid metabolism have long been associated with chronic viral infections[7-13], and the discovery of a specific HCV strain based on genotype 2 (JFH-1; Japanese fulminant hepatitis-1) that efficiently infects and replicates in cultured Huh7.5/7.5.1 hepatoma cells [14-17]has provided considerable insight regarding the intimate link between host cell lipids and HCV infection, at virtually each stage of the HCV replication cycle[11].

However due to lack of genotype based study of COVID and mutations increasing the transmissibility, virulence and mortality of infection finding specific early prognostic marker is of utmost need. With finding of de-arranged glycolytic pathway and β oxidation has led this study to exploration of more accurate early prognostic marker of COVID by ketonuria. Further, correlating disease severity with ketonuria, subjects with severe and moderate ketonuria shown more disease burden and severity. These findings can be alluring ketonuria as prognostic marker for COVID.

Conflict of Interest: None

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