VOLUME - 12, ISSUE - 04, APRIL - 2023 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper

Pathology

UNUSUAL COLOR OF URINE AND SERUM, SHOULD ONE INVESTIGATE THE CAUSE OR NOT?

Dr. Milind Bhide Pathology Services, Girgoan, Mumbai.

Dr. Majethia Nikhil K SRL Diagnostics Dr. Avinash Phadke Labs, Mahim, Mumbai, (Pathologist & Deputy Quality Manager)

Dr. Phadke Ajay A* SRL Diagnostics Dr. Avinash Phadke Labs, Mahim, Mumbai, (Centre Head) *Corresponding Author

ABSTRACT Laboratory diagnosis has become the cornerstone for all clinical diagnosis and treatment, with the advent of genetic workups like DNA sequencing and FISH prognosis of diseases also coming under the umbrella of lab tests. However, the time-tested methods for the analysis of samples before processing (which in the era of accreditation comes under the pre-analytical phase) are still very important in suspecting if not diagnosing disorders apart from preventing pre-analytical errors because of hemolysis, lipemia, QNS, mislabeling, etc. A Yellow serum sample in a rack of tubes is quite common in the laboratory which suggests hyperbilirubinemia. We here like to report a case of 12 years old female child who presented for routine blood and urine testing as she was suffering from low grazed fever, abdominal pain, and loose stool. One look per se the picture favors an infective etiology. But on a collection of specimens the serum turned out to be greenish and the urine too was dark green in color (Fig 1&2) So the questions that we tried to answer by this article are what caused this change in plasma color? what other conditions could cause blood discoloration? And are there concerns or possible interference with laboratory tests? On reviewing the literature, we came across various causes of green discoloration of urine, and interferences encountered routinely which we have discussed in our article. On follow up the child was all right after treatment and has no further episodes of such discoloration.

KEYWORDS : *Biliverdin, BVRA/BLVRA gene, Green Serum, Jaundice.*

INTRODUCTION

The appearance of green serum following centrifugation of a blood sample and urine is very rare. There are, however, several possible causes of this phenomenon. The literature search and the internet have listed three identifiable causes of green serum at least; which are copper compounds, biliverdin/bilirubin, and image dye contrast for radiography. In this case, serum was positive for Hepatitis A IgM antibody, and the next-generation sequencing (NGS) panel (Illumina TruSight One), showed BVRA/BLVRA gene as a cause of hyperbilirubinemia.

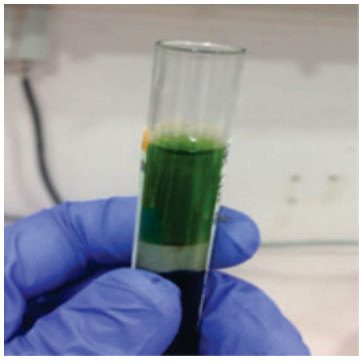

Figure 1: Dark Green discoloration of serum.

Figure 2: Dark Green discoloration of urine

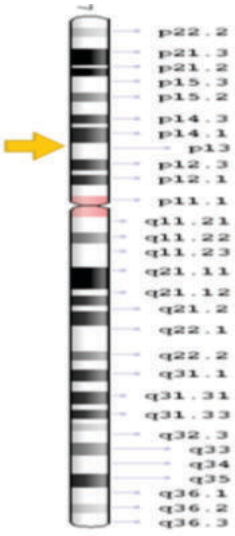

Figure 3: Location of BVRA/BVRB gene on Chromosome 7(Cytogenetic Location: 7p13, which is the short (p) arm of chromosome 7 at position 13 Molecular Location: Base pairs 43,758,122 to 43,807,342 on chromosome 7)

Case History

A 12 years old female child presented for routine blood and urine testing for a low-grade fever, abdominal pain, and loose stool. The plasma sample appeared green in color and the urine appeared in color dark green. No specific diet or medication history.

Lab Results

Table – 1

Haemoglobin	12.5g/dl
WBC	5700/cu.mm
Platelets	329000/cu.mm
Total Bilirubin	2.1 mg/dl
Direct bilirubin	1.93 mg/dl
Indirect bilirubin	0.18 mg/dl

Total Protein	6.1 g/dl
Albumin	3.35 g/dl
SGOT/AST	317 IU/L
SGPT/ALT	1223 IU/L
Alkaline phosphates	541IU/L
ESR:	28 mm/hr;
HAV antibody	Reactive

DISCUSSION

Specimen variations can be controlled through proper patient identification, collection, handling, and strict rejection policies. Light, heat, evaporation, and exposure to the atmosphere will change many substances in routine clinical chemistry testing.

Interference is calculated relative to the measurement of an analyte in a control or base pool. In some cases, the control pool may contain a certain amount of endogenous interferent (i.e., the average concentration of the substance in the patient population from which the pool was obtained). Common examples are bilirubin, hemoglobin, protein, and lipids. Some measurement procedures compensate or correct for the average concentration of interfering substances so that the interference effect is reduced in the patient population. Typical approaches include sample pretreatment, blanking, serum-based calibration, and mathematical correction. Error is introduced when the concentration of interfering substance in the patient specimen is greater than or less than the average concentration in the patient population However attempt to correct analyte results based on CLSI or any study results is not advisable as the relationship between analyte and interferent has not been determined on the instrument at use in the interested setup.

Laboratory interferences such as Bilirubin, in-vitro hemolysis, ascorbate, and lipemia are the most common in biochemistry analysis and these are identified by the naked eye. However, many studies reviewed that icterus interferences are less than 10% up to 8mg/dl of bilirubin, hemolysis is 10upto 5g/dl and lipemia is less than 3% up to 1000mg/dl of intrepid.

Green jaundice has been documented with cases of green serum and green-black urine. A review on green jaundice concluded that "a mixture of biliverdin, mesobiliverdin, and related pigments appeared to contribute to the green color observed."

In over 1000 blood transfusion bottles checked at the Leeds Blood Transfusion Laboratory in 1968, about 1% had green plasma, and a link between the green coloration and women taking combined oral contraceptives was noticed (15/19 from a random sample). The green color was thought to be due to ceruloplasmin, a blue,copper-containing glycoprotein. Elevated levels (35–70 mg/100 mL) were found in these blood samples and it was concluded that "Green plasma has long been known to occur in pregnancy. As estrogens are sometimes effective in elevating ceruloplasmin in patients with Wilson's disease, increased ceruloplasmin in these pill users was probably due to the estrogen in the pill".

At least one report of green coloration of serum has been reported with a fluorescent dye used to evaluate mesenteric-vessel viability by intraoperative angiography in a patient with colon cancer. However, such a history of blood transfusion, copper-containing compound ingestion, or any other drug intake has not been present in our case.

Imaging dyes could theoretically interfere with test results, depending on the detection system used in the test (i.e. colorimetric, fluorescence, or luminescence). However, no radiology studies had been carried out before the blood collection.

Biliverdin is converted to bilirubin in the second step, catalyzed by biliverdin reductase. You can monitor this reaction colorimetric ally in a familiar in situ experiment.

When you are bruised, the black and/or purple color results from hemoglobin released from damaged erythrocytes. Over time, the color changes to the green of biliverdin, and then to the yellow of bilirubin.

More recently, it has been concluded that this green jaundice may be caused by a genetic defect in the biliverdin reductase-A (BVR-A) gene causing reduced activity of the enzyme that converts biliverdin to bilirubin, in conjunction with decompensated liver cirrhosis. In our case serology showed Hepatitis A virus infection and a next-generation sequencing (NGS) panel (Illumina TruSight One), was run on DNA extracted from peripheral blood, using the Illumina HiSeq 2500 platform. Bioinformatic analysis was carried out on coding regions and exon-intron boundaries of the BVRA/ BLVRA gene implicated in hyperbiliverdinemia were identified as BLVRA gene, exon 7 NM_000712.3:c.559T>C NP_000703.2:p.Ser187Pro Homozygous. This is a novel missense variant that, to the best of our knowledge, has never been reported in the literature. In-silico mutation analysis software tools including SIFT, Polyphen, and MutationTaster have classified this variant as 'pathogenic/ disease causing'. Based on available evidence, this variant is classified as a VUS. Biliverdin reductase A (BVR) catalyzes the reduction of biliverdin (BV) to bilirubin (BR) in all cells.

Others and we have shown that biliverdin is a potent anti-inflammatory molecule, however, the mechanism by which BV exerts its protective effects is unclear. Biliverdin (BV) has emerged as a cytoprotective and important anti-inflammatory molecule. Conversion of BV to bilirubin (BR) is catalyzed by biliverdin reductase (BVR) and is required for the downstream signaling and nuclear localization of BVR. Recent data by others and make clear that **BVR is a critical regulator of innate immune responses** resulting from acute insult and injury and that a lack of BVR results in an enhanced pro-inflammatory phenotype. In macrophages, BVR is regulated by its substrate BV which leads to activation of the PI3K–Akt-IL-10 axis and inhibition of TLR4 expression via direct binding of BVR to the TLR4 promoter.

However, we can hypothesize that due to this BLVRA gene mutation normal functioning of biliverdin reductase was hampered and led to the ***accumulation of biliverdin i.e. green jaundice*** **on active infection with Hepatitis A** as confirmed by serology. As the liver function test showed > 20% of fraction as direct bilirubin it indicated a hepatocellular or bilirubin metabolism disorder.

With the sonographic findings, serology workup, and NGS report this was the case of the BLVRA gene, exon 7 NM_000712.3:c.559T>C NP_000703.2:p.Ser187Pro Homozygous mutation. This is a novel missense variant that, to the best of our knowledge, has never been reported in the literature. In-silico mutation analysis software tools including SIFT, Polyphen, and Mutation Taster have classified this variant as 'pathogenic/ disease causing', in this case reducing the liver's ability to metabolize biliverdin to bilirubin in case of Acute Hepatitis A infection causing Green Jaundice.

Visual examination of the specimen may act as a prime indicator of a suspected disorder in this case a genetic disorder. Targeted genetic testing for this variant may be carried out in apparently affected and unaffected family members to assist with phenotypic correlation (i.e., Segregation analysis). Genetic counseling is recommended.

CONCLUSIONS

We can hypothesize that due to this BLVRA gene mutation normal functioning of biliverdin reductase was hampered and this led to the accumulation of biliverdin i.e., green jaundice on active infection with Hepatitis A as confirmed by serology.

As the liver function test showed > 20% of fraction as direct bilirubin it indicated a hepatocellular or bilirubin metabolism disorder.

With the sonographic findings, serology workup, and NGS report this was the case of the BLVRA gene, exon 7 NM_000712.3:c.559T>C NP_000703.2:p.Ser187Pro Homozygous mutation.

Key Messages:

The case of BLVRA gene, exon 7 NM_000712.3: c.559T>C NP_000703.2:p.Ser187Pro Homozygous mutation. This is a novel missense variant that, to the best of our knowledge, has never been reported in the literature.

REFERENCES:

1. Higgins C. A very unusual case of jaundice. The Biomedical Scientist 2009; 53 (10): 828–30.
2. Robert J. McEnroe, Mary F. Burritt, Donald M. Powers, Douglas W. Rheinheimer, MT Brian H. Wallace. Interference Testing in Clinical Chemistry; Approved Guideline— Second Edition Volume 25 Number 27.
3. Bailey H, Wu AHB. Images in clinical medicine. Bright green serum. NEngl J Med 2007; 356 :11: e10.
4. Greenberg AJ, Bossenmaier I, Schwartz S. Green jaundice – a study of serum biliverdin, mesobiliverdinand other green pigments. Am J DigDis 1971; 16 :10: 873–80.
5. Tovey LA, Lathe GH. Caeruloplasmin and green plasma in women taking oral contraceptives, pregnant women, and patients withrheumatoid arthritis. Lancet 1968; 2: 7568: 596–600.
6. Scherer K, Studer W, Figueiredo V, Bircher AJ. Anaphylaxis to isosulfan blue and cross-reactivity to patent blue V: case report and review of the nomenclature of vital blue dyes. Ann Allergy Asthma Immunol 2006; 96: 3: 497–500.
7. Qing H. Meng, Beverly Handy, Elizabeth Wagar. A Case of Green Blood. Clinical Chemistry 2014; 60:4: 695–704.
8. David L. Nelson ,Michael M. Cox Lehninger. Principles of biochemistry, 4th ed.; Chapter 22.
9. Gåfvels M, Holmström P,Somell A et al. A novel mutation in the biliverdin reductase-A gene combined with liver cirrhosis results in hyperbiliver dinaemia (green jaundice). Liver Int 2009; 29:1116–24.
10. Barbara Wegiel, Leo E. Otterbein. Go green: the anti-inflammatory effects of biliverdin reductase. Front Pharmacol. 2012; 3: 47.