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Icteric Plasma in Healthy Donors to Transfuse or to Discard

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A B S T R A C T

Background: Icteric plasma, a yellowish color of plasma caused by elevated levels of bilirubin, is a frequent cause of blood donations especially by Hurricane Gilbert maladies. The significance of the occurrence and clinical consequence of icteric plasma is often surrounded with controversy especially on its safety during blood transfusion.

Methods: It was a rate-based prospective observational study carried out in the Ruxmaniben Deepchand Gardi Medical College, Ujjain Blood Bank, between December 2017 and June 2019. The purpose of the study was to establish the occurrence of mild hyperbilirubinemia in otherwise-healthy blood donors. Whole blood, tested as sero-negative, was screened to icteric plasma, and several types of biochemical tests were performed, which include the liver functions tests, and markers of hemolysis.

Results: A total of 8,971 donations were examined during 18 months period, and 66 icteric bags were registered (1.355). Icteric plasma was highest among the 26-35 years group (62.1%) age group and lowest among 0-15 years group (0.5%). Confirmatory tests by biochemists revealed that although the total bilirubin was high, the rest of the profiles were normal inclusive of liver enzymes, hemoglobin, and protein levels hence the plausible benign nature of the discoloration.

Conclusion: The majority of the population that has because of Gilbert syndrome the problem of icteric plasma is really a major problem in the blood donation process, whereby the greatest in this age group/range is between the age groups of 26-35 years. Although there are already regulatory guidelines regarding the rejection of icteric plasma the present study allows to rethink the policies concerning re-use of icteric plasma, which does not seem to produce any serious risk to the recipient, at least when no liver dysfunction, hemolysis, or infections are present.

Keywords: Gilbert syndrome, Icteric Plasma, Hyperbilirubinemia, Blood Transfusion, Blood Donation.

INTRODUCTION

Blood transfusion is a life-saving therapeutic procedure whose quality control has to be very strict to make it safe to both the donor and the recipient[1]. Visual inspection of plasma components is one of the quality control parameters that are used in the process of preparing blood components[2]. The most frequent problem that is frequently observed in blood banking practice is the occurrence of icteric or yellowish plasma in the donation of apparently healthy blood donors, which poses a clinical dilemma on the safety and acceptability of such components to be used in transfusion[3,4].

Icteric plasma is a yellow to greenish discolouration caused by increased bilirubin levels, and it is found in 0.19 to 0.99 percent of blood donations by healthy volunteers[5,6]. The rate of such phenomenon differs greatly in various population and geographical areas with the rates reported as 0.71 percent to 6.3 percent of

all donations[4,7]. This disparity can be explained by the variability in donor population, the genetic predisposition, and the existing metabolic diseases that influence the metabolism of bilirubin[8].

Gilbert syndrome, a benign genetic disorder that has a prevalence of 3-7 percent in the general population (and is more common in men than in women[9,10], is the main cause of icteric plasma in healthy blood donors. Gilbert syndrome is a mild unconjugated hyperbilirubinemia caused by low activity of the bilirubin uridine diphosphate glucuronosyltransferase (UGT 1 A 1) enzyme[11]. The condition shows autosomal recessive transmission and is related to polymorphism in UGT1A1 gene promoter region, especially TA repeat polymorphism[12,13]. Although being benign, the Gilbert syndrome may result in different levels of bilirubin (1.2-5.0 mg/dL) that is enough to discolor the plasma[14,15].

The general advice of the current regulatory guidelines by various international bodies such as World Health Organization, Food and Drug Administration and the European blood banks is that blood components with abnormally colored plasma should not be used[16,17]. The JPAC guidelines have specifically indicated that platelet and plasma components should not be derived out of icteric donations[18]. Precautionary principle is the main basis of these recommendations, as they are designed to eliminate possible transfer of infectious diseases or metabolic disorders that can be expressed in the form of hyperbilirubinemia[19].

The wholesale rejection of icteric plasma constituents by donors with Gilbert syndrome is however a major challenge to blood banking services especially in resource-constrained settings where shortage of blood is the order of the day[20,21]. Research has shown that the donors with Gilbert syndrome would show isolated unconjugated hyperbilirubinemia without signs of hemolysis, hepatic dysfunction, or infectious disease parameters[22,23]. Laboratory analysis of these donors is repeatedly normal with normal liver enzymes, negative viral serologies and the lack of hemolytic markers, indicating that the discoloration of the plasma is due only to the benign metabolic disorder[24,25].

The safety of transfusion of plasma components of donors with Gilbert syndrome has been an area of great controversy in the transfusion medicine fraternity. Hypothetical issues on the possible danger to the recipients (especially neonates and pre-existing liver disease affected patients) have contributed to the cautious applications in the use of the components[26]. Mathematical modeling experiments have indicated that the extra burden of bilirubin in the case of transfused plasma would cause insignificant elevations of the serum bilirubin level even in frail groups like premature babies[27]. As an example, a 1-kg premature baby who consumes 10 ml of plasma containing 3 mg/dL of bilirubin would have an estimated increase of only 0.6 mg/dL in the serum bilirubin concentration which would, in addition, be further counteracted by extravascular diffusion[1].

The economic cost of throwing away the icteric plasma components is high especially when the prevalence of Gilbert syndrome is high in some population groups. According to research conducted in different blood centers, icteric plasma is responsible of discarding between 0.71 percent and 6.3 percent of donations, which is a major loss of potentially safe blood components[4,28]. This wastage is especially critical in those countries with low rates of voluntary donation and high positivity rates of transfusion-transmitted infections where every safe unit is precious in terms of ensuring sufficient blood stock[29].

Newer donor screening and laboratory testing has improved the capacity to distinguish benign causes of hyperbilirubinemia, e.g. Gilbert syndrome, and pathologic conditions necessitating donor deferral[30]. Extensive screening procedures such as complete blood count, liver functions, lactate dehydrogenase, and hemolytic markers can be used to screen donors with Gilbert syndrome and rule out other plasma

discoloration factors[31,32]. Also, genetic analysis of UGT1A1 polymorphism has become a certain diagnostic method to determine the presence of Gilbert syndrome in donors with icteric plasma[33].

No adverse event of special concern directly related to transfusion of plasma components collected by donors with Gilbert syndrome, which were accidentally released to clinical use, has been reported in the hemovigilance data of different countries[34]. This lack of reported complications with the increasing volume of evidence to support the safety of these components has led to the demand of re-evaluation of the existing regulatory guidelines[35,36]. Some authorities in transfusion medicine have argued in favour of the creation of evidence-based policies which would enable the conditional usage of icteric plasma components of donors with confirmed Gilbert syndrome, especially in regard to certain clinical indications where the advantages would supersede the hypothetical dangers[37].

Ethical concerns that exist in regards to discarding of safe blood components should also be assessed within the framework of global blood shortage and beneficence and non-maleficence in the medical practice[38]. Universal discarding as it is currently practiced could be interfering with patient care in some instances where an urgent need of blood components will be required, and at the same time will not be able to deliver any quantifiable safety advantages to the recipients[39].

MATERIALS AND METHODS

The prospective observational study was done in the Ruxmaniben Deepchand Gardi Medical College (RDGMC) Blood Bank, Ujjain, during the 18 months period between December 2017 to June 2019. The research was conducted in order to determine the frequency of mild hyperbilirubinemia among healthy blood donors, who were sero-negative. The study was ethical and written informed consent was obtained by all the donors prior to their participation in the study.

Any donor who was willing was screened as per the guidelines of National AIDS Control Organization (NACO), and then a pre-donation medical examination was carried out to determine eligibility. The NACO criteria were met by the donors aged between 18-60 years with a hemoglobin level of 12.5 gm/dl or above and blood samples were taken. Whole blood of 450 ml was taken in CPDA or SAGM blood bags. Blood samples were also tested towards the required Transfusion Transmitted Infections (TTI) screening by enzyme-linked immunosorbent assays (ELISA) on HIV, HBV, HCV, syphilis and malaria.

Blood Sample Collection and Analysis

In the study, sero-negative blood bags were only chosen. The color of plasma appeared on the 3 rd or 4 th day following the collection, and the bags with icterus were analyzed further. Several biochemical parameters were determined by the VITROS 250 analyzer such as liver function test (levels of bilirubin, SGOT, SGPT, alkaline phosphatase, GGT, total proteins, albumin, globulin, and A/G ratio). The blood of the donor was also examined by complete blood count (CBC), peripheral smear, reticulocyte count, G6PD test, and indirect Coombs test.

Inclusion and Exclusion Criteria

Whole blood donations within 18 months, blood bags with less than five days, and a volume of 450 ml were the inclusion criteria, and those that were collected to prepare components, blood bags exceeding five days, and those that tested positive to any TTI were the exclusion criteria.

Statistical Analysis

Descriptive statistics were computed and statistical tests used to obtain significant correlations between biochemical markers and icteric plasma.

RESULT

Table 1: Whole Blood Donation Over Period of 18 Months and Incidence of Icteric Bags

Month	Total Number of Donor Collected	Elisa Positive Cases	Icteric Bags	Bags in %
January 2018	447	10	2	0.32
February 2018	405	3	5	0.40
March 2018	442	7	4	0.11
April 2018	363	7	6	0.57
May 2018	591	7	2	0.80
June 2018	597	8	5	0.42
July 2018	489	16	6	0.45
August 2018	650	7	5	0.42
September 2018	479	10	5	0.42
October 2018	625	14	6	0.44
November 2018	478	13	5	0.43
December 2018	374	7	6	0.48
January 2019	452	10	4	0.31
February 2019	384	14	3	0.26
March 2019	313	4	6	0.56
April 2019	495	8	4	0.37
May 2019	522	5	4	0.34
June 2019	865	13	5	0.39
Total	**8971**	**153**	**66**	**1.35**

This table provides a comprehensive overview of whole blood donations over a period of 18 months (from January 2018 to June 2019). It shows the total number of donors collected each month, the number of Elisa positive cases, and the number of Icteric bags recorded. Additionally, it includes the percentage of Icteric bags relative to the total donations for each month. For example, in January 2018, there were 447 total donors, 10 Elisa positive cases, and 2 Icteric bags, representing 0.32% of the donations. The data reveals fluctuations in the incidence of Icteric bags, with a total of 66 Icteric bags across 8971 donations, accounting for 1.35% overall.

Table 2: Distribution amongst Various Age Groups

Age Groups	Frequency	Percent
<= 25 years	9	13.6
26 - 35 years	41	62.1
36 - 45 years	10	15.2
> 45 years	6	9.1
Total	**66**	**100**

Table 2 outlines the distribution of Icteric bags among different age groups. The age groups are divided as follows: ≤ 25 years, 26-35 years, 36-45 years, and > 45 years. The frequency and percentage of Icteric bags within each age group are listed. The largest number of Icteric bags was observed in the 26-35 years age group, which accounted for 62.1% of the cases. This was followed by the 36-45 years group with 15.2%, the ≤ 25 years group with 13.6%, and the > 45 years group with 9.1%. Overall, the table highlights the prevalence of Icteric bags across age groups, with the majority being found in the 26-35 years range.

Table 3: Mean Values for Various Parameters

Variable	Mean	Standard Deviation	Standard Error of Mean	Percentile 05	Percentile 95	Minimum	Maximum
Hb	14.16	1.16	0.14	12.50	16.00	12.50	16.70
BIL total	2.01	0.39	0.05	1.40	2.71	1.40	3.01
Bil direct	0.53	0.27	0.03	0.00	1.00	0.00	1.40
Bil Indirect	1.44	0.42	0.05	0.65	2.00	0.53	2.70
SGOT	30.27	8.58	1.06	22.00	48.00	21.00	72.00
SGPT	38.17	8.74	1.08	26.00	51.00	23.00	84.00
GGT	31.59	5.89	0.73	22.00	40.00	21.00	42.00
ALP	56.48	16.24	2.00	31.00	80.00	31.00	107.00
Total Protein	6.53	0.70	0.09	5.20	7.79	4.70	7.98
Alb	3.65	0.46	0.06	2.90	4.40	2.50	4.90
Glb	2.86	0.36	0.04	2.31	3.49	2.10	3.80
A/G ratio	1.31	0.17	0.02	1.01	1.60	0.90	1.64

Table 3 summarizes the mean values for various laboratory parameters across all age groups. It includes data for hemoglobin (Hb), bilirubin levels (total, direct, and indirect), liver enzymes (SGOT, SGPT, GGT, ALP), total protein, albumin, globulin, and the albumin/globulin (A/G) ratio. The table also provides the standard deviation, standard error of mean, percentiles (5th and 95th), as well as the minimum and maximum values for each parameter. For instance, the mean hemoglobin level across all age groups is 14.16 g/dL with a standard deviation of 1.16, while the mean total bilirubin is 2.01 mg/dL with a standard deviation of 0.39. This table serves as a comprehensive guide for understanding the distribution of key clinical parameters in healthy blood donors.

Table 4: Variable Data for Age Group <= 25 Years

Variable	Mean	Standard Deviation	Standard Error of Mean	Percentile 05	Percentile 95	Minimum	Maximum
Hb	14.36	1.34	0.45	12.50	16.00	12.50	16.00
BIL total	2.12	0.44	0.15	1.40	2.75	1.40	2.75
Bil direct	0.46	0.32	0.11	0.00	1.00	0.00	1.00
Bil Indirect	1.62	0.34	0.11	1.00	2.00	1.00	2.00
SGOT	33.44	9.32	3.11	23.00	56.00	23.00	56.00
SGPT	37.33	7.58	2.53	25.00	48.00	25.00	48.00
GGT	32.00	5.27	1.76	24.00	40.00	24.00	40.00
ALP	55.89	11.66	3.89	45.00	80.00	45.00	80.00
Total Protein	6.53	0.45	0.15	5.99	7.17	5.99	7.17
Alb	3.70	0.27	0.09	3.30	4.10	3.30	4.10
Glb	2.84	0.30	0.10	2.43	3.28	2.43	3.28
A/G ratio	1.38	0.13	0.04	1.18	1.60	1.18	1.60

This table provides detailed data for the ≤ 25 years age group, showing the mean values for various biochemical parameters including Hb, total bilirubin, SGOT, SGPT, GGT, ALP, total protein, albumin, globulin, and A/G ratio. For this age group, the mean hemoglobin level is 14.36 g/dL with a standard

deviation of 1.34, and the mean total bilirubin level is 2.12 mg/dL. Liver enzyme levels (SGOT, SGPT, GGT, and ALP) also vary within specific ranges, with SGOT having a mean value of 33.44 U/L. The table additionally provides the standard error of mean, percentiles, and the minimum and maximum values for each variable, offering insights into the biochemical characteristics of blood donors within this age group.

Table 5: Variable Data for Age Group 26 - 35 Years

Variable	Mean	Standard Deviation	Standard Error of Mean	Percentile 05	Percentile 95	Minimum	Maximum
Hb	14.30	1.04	0.16	12.50	15.60	12.50	16.70
BIL total	1.99	0.38	0.06	1.50	2.70	1.40	3.00
Bil direct	0.52	0.23	0.04	0.18	0.80	0.10	1.00
Bil Indirect	1.42	0.40	0.06	0.90	1.92	0.60	2.70
SGOT	29.27	6.88	1.07	22.00	40.00	21.00	55.00
SGPT	37.37	9.55	1.49	26.00	46.00	23.00	84.00
GGT	31.68	5.91	0.92	22.00	40.00	21.00	42.00
ALP	55.83	12.16	1.90	33.00	77.00	31.00	80.00
Total Protein	6.52	0.74	0.12	5.50	7.79	4.70	7.98
Alb	3.62	0.50	0.08	2.90	4.40	2.50	4.90
Glb	2.89	0.38	0.06	2.37	3.49	2.10	3.80
A/G ratio	1.31	0.18	0.03	1.01	1.60	0.90	1.64

Table 5 outlines the variable data for the 26 - 35 years age group, detailing the mean values of various clinical parameters. The mean hemoglobin level for this group is 14.30 g/dL with a standard deviation of 1.04, and the total bilirubin level is 1.99 mg/dL. The liver enzymes (SGOT, SGPT, GGT, and ALP) reflect slightly lower values compared to the ≤ 25 years group, with SGOT averaging 29.27 U/L. The table also includes the standard error of the mean, percentiles, and the minimum and maximum values for each parameter. This data provides a clear understanding of the health status of donors in the 26-35 years age group, which is the most frequent group for Icteric bags, as seen in Table 2.

DISCUSSION

The results of the present study on the icteric plasma in blood donations demonstrate valuable trends that are consistent with and contrast with the existing literature. The rate of 1.35 percent of overall donations, which was recorded in this 18-month study, is in line with the rates reported in other studies though it is a higher rate than some similar studies.

Comparisons of Incidence Rates in Studies

The incidence rate of 1.35 percent in this study is much higher than a number of other studies. Arora et al. reported a five-year prevalence of asymptomatic hyperbilirubinemia in healthy donors of 0.91 percent [40], whereas Kumar et al. identified Gilbert syndrome in 0.71 percent of donations[41]. These rates are however significantly lower than those found by Kumar et al who indicated that 6.3 percent of the donations exhibited icteric plasma[42]. Such difference in the incidence rates between studies can be explained by several factors, such as geographical location, donor demographics, screening protocol and seasonal differences in the donation patterns.

A more recent study by researchers who studied altered color plasma components found an incidence of all color alterations of 0.19 percent, of which yellow discoloration was only a subset[43]. This 0.19 percent rate, which is so much less than that of 1.35 percent in the present study, indicates that there may be a wide regional or methodological disparity in both detection and reporting of cases of icteric plasma.

The result of the present study that the age group of 26-35 years had the highest occurrence (62.1%) of icteric bags has a significant agreement with the available literature on prevalence of Gilbert syndrome. According to Kumar et al., the mean age of donors with Gilbert syndrome was 27.92 +/- 7.19 years, and the youngest was 19 years and the oldest was 52 years[41]. This is very much in line with the highest incidence of the 26-35 years age group in the present study.

This age distribution pattern is also in line with the epidemiology of Gilbert syndrome that is known to occur during puberty because of the effect of hormones on bilirubin metabolism[44,45]. The syndrome is prevalent among about 5 percent of the general population in the United States and more men than women are diagnosed of the syndrome[45]. This can be attributed to the genetic predisposition as well as the physiological conditions that exist at this stage of life.

The average total bilirubin of 2.12 mg/dL found on the 26-35 years age group in this study is relatively consistent with that of the literature on Gilbert syndrome. Kumar et al. reported a mean bilirubin levels in the serum of 1.86 +/- 0.57 mg/dl (range: 1.21-3.5) among donors with confirmed Gilbert syndrome[41]. In the same way, the serum total bilirubin levels were found to be 1.6 to 3.1 mg/dL with a mean level of 2.19 mg/dL as reported by Arora et al.[40].

The results of liver enzymes in the present study, which demonstrated high level of SGOT and SGPT in icteric patients, are consistent with the earlier reports. But these elevations are normally in acceptable limits of blood donation as shown by Kumar et al., who recorded mean aspartate aminotransferase of 24.8 +/- 7.53 IU/L and alanine aminotransferase of 26.48 +/- 10.99 IU/L in donors having Gilbert syndrome[41].

The monthly variations, which are recorded in this research with the highest incidence in May 2018 (0.8%) and the lowest in February 2019 (0.26%), are a new discovery that has not found much coverage in the blood banking literature. Although the seasonal change in blood donation rates has been reported and the studies indicated that the donations declines during the holiday season and rise during certain awareness campaigns[46], the seasonal change in icteric plasma incidence has not been well explored.

Other studies have been pointing to seasonal trends in hepatitis and liver related diseases, where the highest numbers are usually recorded during spring and summer[47]. This seasonal trend might be the reason behind the changes in icteric plasma incidence since the change in the environment, dietary habits, and physiological stress in the different seasons might interfere with the bilirubin metabolism.

Different rates of incidence of icteric plasma in blood donations have been reported in studies conducted internationally. In their classic report, Naiman et al. determined icteric plasma in the donated blood components and determined that all seven donors were identified to have Gilbert syndrome, a disorder that occurs in 3-5 percent of the general population[48]. The current findings are supported by this study that has established the close relationship between icteric plasma among healthy donors and Gilbert syndrome.

The prevalence rates have been found to be different in European studies with some being higher in certain populations. The differences in the reported rates in various geographical locations could represent genetic variations in the enzyme activity of UGT1A1, diet or differences in donor screening procedures[44].

The results of the present research on the safety profile of donors with icteric plasma are in line with the literature proving the harmless character of Gilbert syndrome. It has always been revealed that with isolated unconjugated hyperbilirubinemia in donors with Gilbert syndrome, liver functions tests are normal, no hemolysis, and negative serology of viruses[40,48,41].

Studies involving mathematical modeling have indicated that despite being a vulnerable group, premature infants, the extra load of bilirubin due to the infusion of plasma that contains high levels of bilirubin would have minimal clinical significance[42]. This confirms the emerging belief that the universal disposal of icteric plasma constituents might just be a waste of valuable blood resources.

The incidence rate of 1.35 percent, as recorded in this study, has a great economic implication to the blood banking services. In comparison with the results of Kumar et al., who found that 0.71 percent of donations were discarded because of icteric plasma[41], the present study implies the possibility of even more wastage of resources. This is especially worrying in resource-constrained environments where shortages of blood are regular and each safe unit is precious towards ensuring a sufficient stock.

CONCLUSION

The present study shows the high incidence of icteric plasma in healthy blood donors, Gilbert syndrome, an explanation of mild unconjugated hyperbilirubinemia. The reported frequency of the cases was 1.35% and most cases were seen in the 26-35 years demographic interval (62.1%). Biochemically, the high levels of bilirubin were observed but all other indicators including liver enzymes, hemoglobin, and proteins were on normal levels implying that the plasma discoloration was attributed to the harmless ailment known as Gilbert syndrome. The evidence is in favor of the idea that the transfusion of plasma of the donors with Gilbert syndrome is not of great threat to the recipients without signs at the presence of liver diseases, hemolysis, and infection despite the precautionary advice that is against the usage of the icteric plasma. These findings confront the categorical refusal of the use of icteric plasma and the importance of evidence-based recommendations according to which it is possible to use such plasma in some circumstances, in particular during shortages of blood.

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