# Evaluation of immunoglobulins IgG, IgM, IgA and Complement Components C3 and C4 Levels in Iraqi full term Neonates with Severe Hyperbilirubinemia

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## Abstract

**Background and aim:** Hyperbilirubinemia is an elevation of the bilirubin levels in blood of newborn babies that presented as jaundice. This study aimed to evaluate the levels of important of immunological system proteins (IgG, IgM, IgA, C3 and C4) in both newborn babies with severe hyperbilirubinemia and healthy controls.

**Methods**: Thirty (30) full-term neonates with severe hyperbilirubinemia (serum bilirubin >20 mg/dl) and twenty five (25) healthy control full-term neonates were included in the study. Blood samples taken and Hb, total serum bilirubin, total protein measured immediately by spectrophotometer. IgG, IgM, IgA, C3 and C4 measured by immune-nephelometric method.

**Results:** In hyperbilirubinemia group (Male/ Female percentages) were (63% / 37%), (IgG 373.4±218 mg/dl),( IgM 20.5 ± 24.03 mg/dl), and (C3 209.4±17.81 mg/d)l values were significantly lower than control group ( 588.2±298.5 mg/dl IgG, 26.44 ± 2.92 mg/dl IgM and 627.5±221.1 mg/dl for C3). Correlation analysis revealed that in control group IgG, IgM, and C3 shown to be significantly (P value <0.05) and positively correlated (r-value > 3) with both age and weight.

**Conclusions:** jaundice is a risk factor for sepsis that could be due to lower levels of immunoglobulins and complements components. The normal development of immune system that observed with increasing age and weight was impaired in patients with neonatal hyperbilirubinemia.

***Key words:*** *Neonatal hyperbilirubinemia, Complement components, Immunoglobulins and Total serum bilirubin.*

## Introduction

Hyperbilirubinemia is one of the commonest clinical conditions in newborn babies that presented as jaundice [(1)]. It affects both full-term and premature neonates in about 60% and 80% respectively [(2, 3)]. However, most of the cases are physiological jaundice that is a safe and do not involve any clinical complications, but excessive elevation of unconjugated bilirubin has a potential of development of neurotoxicity and kernicterus. A variable risk factors have been proposed for neonatal hyperbilirubinemia that includes; genetic, maternal, prenatal, neonatal and other factors [(4)].

Physiologically, bilirubin is produced and elevated in neonatal life as a results of excessive distraction of fetal red blood cells [(5)]. These resultant molecules are not merely bi products of a heam metabolism rather than its potential cytoprotective role and has an antioxidant activity such as that observed of serum uric acid [(6, 7)]. Newborn baby is continuously produced free radicals even in the absence of any pathology , during this period the body is at great vulnerability of oxidative stress [(8)]. Indeed, at this period different body antioxidant mechanisms are not fully matured and thus bilirubin at

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physiological level is suggested as a defense mechanism against the harmfully generated reactive oxygen species [9]. The other side it showed that exceeding limited level of bilirubin in neonatal period has an adverse effect on baby defense mechanisms. Studies had shown that a strong associations were observed between hyperbilirubinemia and risk of infection and septicemia [10, 11]. Furthermore, accumulative evidences have demonstrated that, severe hyperbilirubinemia was associated with modulation of different components of immune response. Haga, Tempero [12] showed that intracellular bilirubin precipitation causes an inhibitory effect on T helper-1 cell and their inflammatory response. Neonates with hyperbilirubinemia showed an impairment of immunological response (decrease immunoglobulins production) to routine vaccination series against measles, diphtheria and tetanus [13]. Neonates with hyperbilirubinemia showed a reduced Lymphocytes proliferation [14].

Both adaptive and innate immune responses are still immature during neonatal period [15]. B lymphocytes, the origin of immunoglobulins, produced in late 1st trimester in fetal life but remain unable to produce antibodies or immunoglobulin class switching during early neonatal periods [16]. Indeed, active IgG production starts around the third month of life but during neonatal period serum IgG represents maternal IgG transported via placenta to fetal blood. IgA in neonatal serum supplied via early breast feeding [17]. Furthermore, studies observed that the components of complement system are under-developed in newborns as well [18]

Hyperbilirubinemia causes reduction of antibodies production, total, IgM and IgA levels [14]. In addition, a strong inverse association was observed between elevated serum bilirubin and the development and maturation of complement cascade [19].

This study was set to evaluate the influence of sever hyperbilirubinemia in neonates, regardless its cause, on immunological markers represented by IgG, IgA, IgM and complement components C3 and C4.

## Patients and Methods

The current cross-sectional study approved and conducted in Chemistry and Biochemistry Department/ College of Medicine / Mustansiriyah University in 2019-2020. Thirty(30) full-term neonates were selected as cases with severe hyperbilirubinemia (serum bilirubin >20 mg/dl) in neonatal word during their preparation for phototherapy or exchange transfusion in the Central Child Teaching Hospital in Baghdad over the period from April - November 2019. Twenty-five (25) healthy control full-term neonates been chosen from an outpatient clinic of the same hospital for any presentation and shown not have jaundice as confirmed by total serum bilirubin (TSB) measures. For both groups, age, sex, gestational age and mother age recorded and body weight measured. Five (5 ml) of venous blood were with drowning of each individual and allowed to clot, or with drowning in heparin tubes or EDTA tubes. Hb, total serum bilirubin (TSB) and total serum protein measured immediately. Serum and plasma were stored at -20° C or tested immediately after collection and separation for total immunoglobulin (IgG, IgM, IgA, C3 and C4) measurements.

**IgG, IgM, IgA and complement components C3 and C4 measurements:**

Immunoglobulins and complement C3 and C4 measurements performed using nephelometric method; the device used for the assay was Beckman IMMAGE® Immunochemistry Systems using provided commercially available kits by Beckman Coulte, USA. The test performed according to the manufacturer's protocol under controlled laboratory conditions. Note; no interference was observed between serum bilirubin and parameters measured.

## Statistical Analysis

Analysis of data carried out using the available statistical package of Graph Pad Prism (California) software. Data were presented in simple measures of frequency, percentage, mean, standard deviation, and range (minimum-maximum values). The significance of difference of different means (quantitative data) was tested using Students-t-test for difference between two independent means or ANOVA test for difference among more than two independent means. The significance of difference of different percentages (qualitative data) was tested using Pearson Chi-square test ($\chi^2$ -test). Statistical significance was considered whenever the P value was equal or less than 0.05.

## Results

Hyperbilirubinemia affects male (63%) baby more than female (37%). The mean age jaundiced baby was (4.07±1.31) days and their TSB was (23.63±2.30 mg/dl) while (0.9± 0.24 md/dl) in control group, as shown in (Table 1). The mean levels of IgG in control group (588.2±298.5 mg/dl) was significantly higher (P value <0.01) than that found in hyperbilirubinemic babies (373.4±218 mg/dl), the rest of immunoglobulins and complement proteins results were showed in (Table 2) and (Figure 1). IgG, IgM, and C3 shown to be significantly (P value **<0.05)** and positively correlated (r-value > 3) with both age and weight of healthy control neonates as shown in (Figures, 2 and 3) where graphs representative of correlation analysis of neonatal age and weight with IgG, IgM and C3 in both controls and hyperbilirubinemic group.

**Table 1: Characteristics of neonates with hyperbilirubinemia and controls.**

| | | Severe Hyperbilirubinemia | Controls | P value |
|---|---|---|---|---|
| | | No. | No. | |
| | | 30 | 25 | |
| Age (days) | Mean±SD (Range) | 4.07±1.31(2-7) | 3.84 ±1.6 (2-7) | Ns* |
| Gender | Male | 19(63%) | 16 (64%) | Ns* |
| | Female | 11(37%) | 9 (36%) | Ns* |
| | | | | |
| Weight (gm) | | 2675±578.5 (1700-4000) | 3383±526.9 ( 2240-4180) | < 0.0001 |
| Gestational age | | 37.97±1.54 (36-40) | 38.76±1.56 (36-40) | Ns* |
| Mother age (years) | | 27.17±5.16 (19-37) | 27.32±5.35 (19-37) | Ns* |
| Hb (gm/dl) | | 17.33±1.47 (14-20) | 16.88±1.59 (13.4-19.7) | Ns* |
| TSB (mg/dl) | | 23.63±2.30 (20-29) | 0.9±0.24 (0.6-1.32) | < 0.0001 |
| Total serum protein (gm/dl) | | 5.32±0.79 (4.1-6.8) | 6.21±0.73 (5.01-7.48) | < 0.0001 |

*Significant difference between two independent means using Students-t-test at 0.05 level.

**Table 2. Immunoglobulins and complement C3 and C4 for study subjects**

| | | Severe Hyperbilirubinemia | Controls | P value |
|---|---|---|---|---|
| | | No. | No. | |
| | | 30 | 25 | |
| IgG (mg/dl) | Mean±SD (Range) | 373.4±218 ( 81-827) | 588.2±298.5 (167-1308) | < 0.01 |
| IgM (mg/dl) | | 20.5 ± 24.03 (6-104) | 26.44 ± 2.92 (9-121) | Ns* |
| IgA (mg/dl) | | 10.1±5.9 (3-28) | 6.5±5.5 (1-21) | <0.05 |
| C3 (mg/dl) | | 209.4±17.81 (168-236) | 627.5±221.1 (216-1118) | <0.0001 |
| C4 (mg/dl) | | 82.1±25.24 (46-140) | 186±71 (70-396) | <0.0001 |
| *Significant difference between two independent means using Students-t-test at 0.05 level. | | | | |

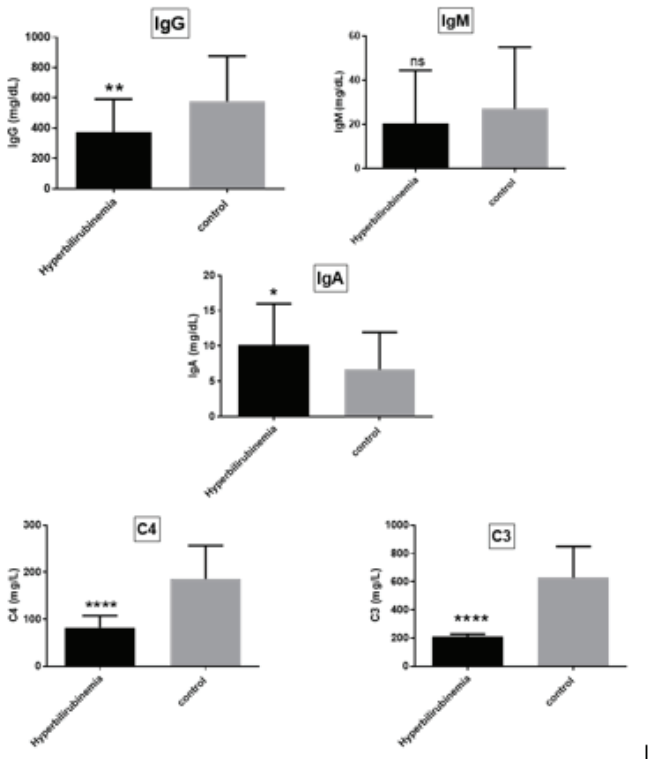


**Figure 1: Immunoglobulins and complement C3 and C4 for study subjects.**

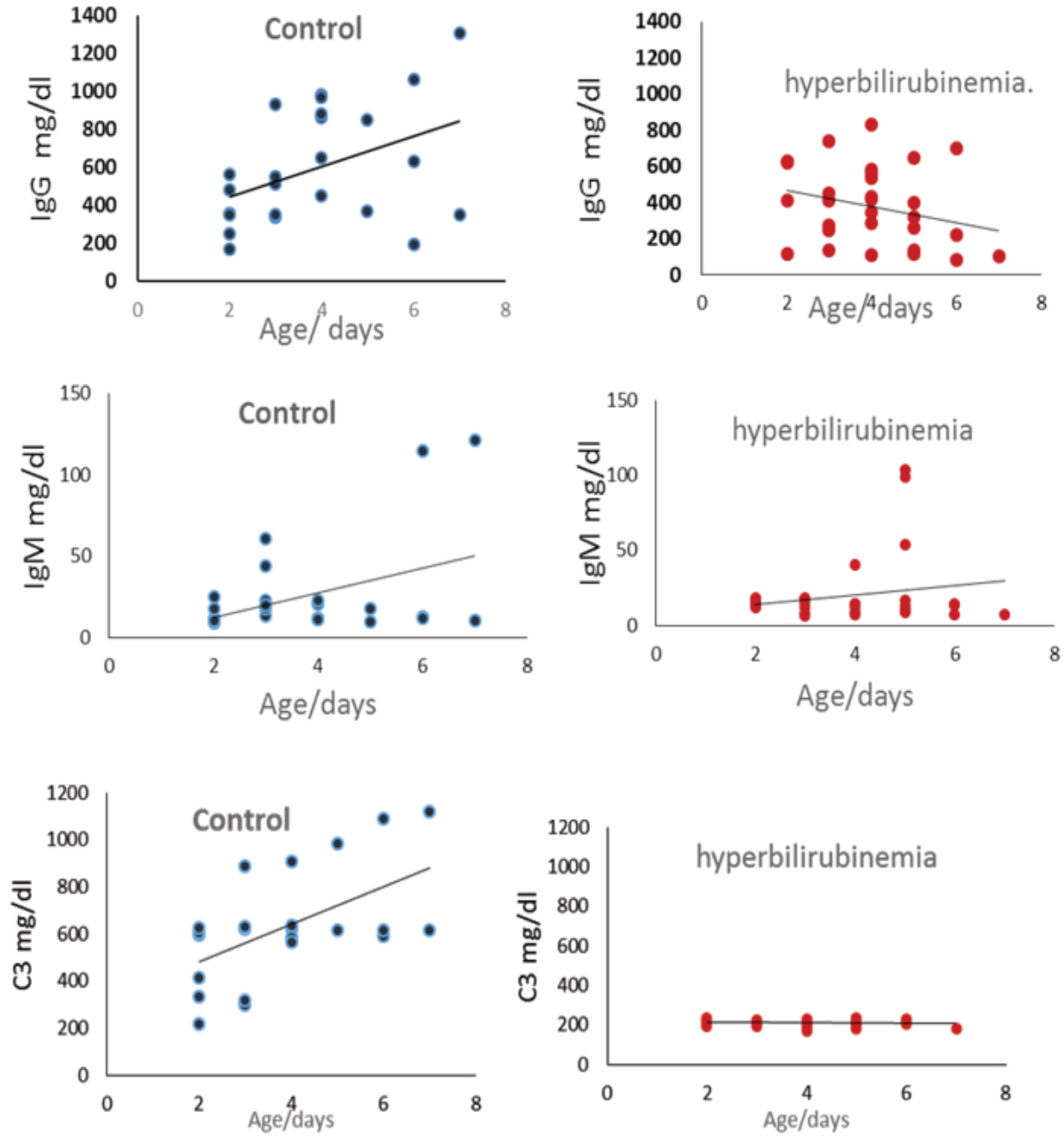


**Figure 2: Gragh representitive of correlation analysis of neonatal age in days with IgG, IgM and C3 in both control and hyperbilirubunemic groups.**

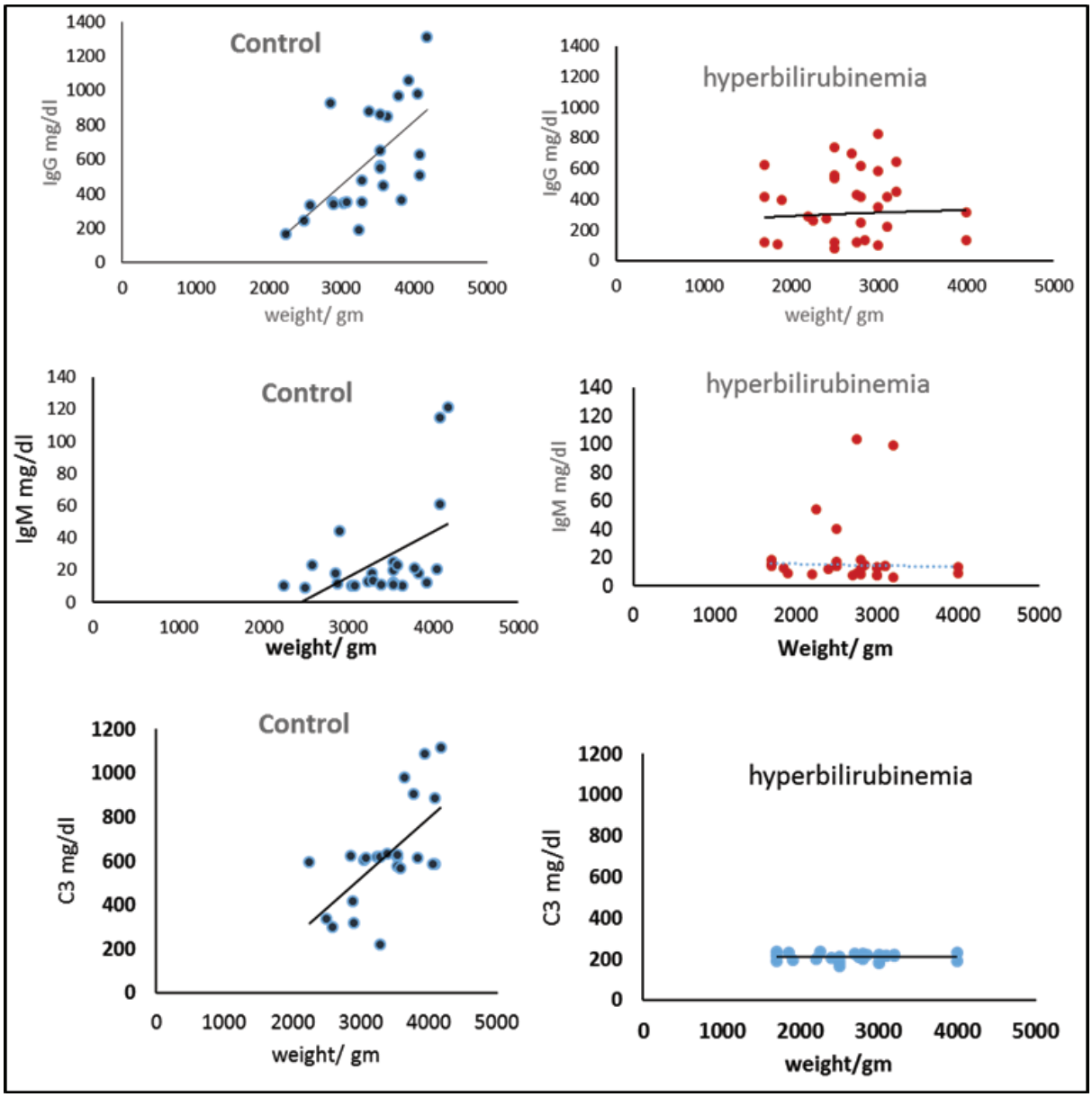


**Figure 3: Gragh representitive of correlation analysis of neonatal weight in grams with IgG, IgM and C3 (mg/dl) in both control and hyperbilirubunemic groups.**

## Discussion

This study aimed to compare the levels of immunoglobulins and complement components C3 and C4 in neonates with severe jaundice, regardless its cause, with aged matched healthy controls.

The main findings of this study were predicted that; in hyperbilirubinaemic group, there were lower body weight and lower total protein level when compared to controls. In addition, low levels of IgG, C3 and C4, while high IgA levels were found in jaundiced neonates compared to controls. No significant differences were demonstrated of level of IgM between the two groups. Furthermore, the strong positive correlation that observed in control group between body weight and age with each of IgG, IgM and C3 was disturbed in babies with hyperbilirubinemia.

Hyperbilirubinemia in newborn babies is usually due to elevation of unconjugated (indirect) bilirubin that accrued as results of increased bilirubin production over conjugation and hepatic clearance of bilirubin [20]. The clinical classification of significant hyperbilirubinemia could categorize according to bilirubin level as;

Significant hyperbilirubinemia (TSB ≥12 ) mg/dL, Severe hyperbilirubinemia (TSB ≥20) mg/dL. Extreme hyperbilirubinemia typically (TSB ≥25) mg/dL and hazardous or critical hyperbilirubinemia (TSB ≥30) mg/dL [1]. In the current study the hyperbilirubinaemic neonates group was either severe or extreme class, TSB (23.63±2.30) mg/dl , the range of TSB readings was (20-29) mg/dl, those admitted to neonatal word for either phototherapy or exchange transfusion treatment.

Results of the current study showed that, neonatal hyperbilirubinemia affects male more than female, more than 60% of hyperbilirubin group were male and they tend to have higher serum bilirubin level (24.1±2.4 )mg/dl than female (22.8±2.0 )mg/dl however this was statistically not significant. These results come in agreements with many studies including, Al-Banna, Riad [21]and Greco, Arnolda [22]. The exact causes for this gender differences are unknown but different mechanisms been suggested. Tioseco, Aly [23] attributes the sex difference due to that dysfunction of placenta, which is more common during pregnancy in male fetus which could contribute to higher risk of early life jaundice. Furthermore, during fetal life, male fetus has higher metabolic rate than female which could contribute to the elevated turnover of bilirubin in male newborn baby [24].

Both Immunoglobulins and complements components are proteins that were detected in the plasma and body fluids and the play a significant role in protection from infections. and the knowledge of these components of immune system in neonates is vital in detection of immunological as well as infectious disease [25].

In control group, the level of IgG and IgM were comparable with study conducted by Kardar, Oraei [26]. They found that mean IgG was (507.6 mg/dl, IgM 26 mg/dl, IgA 5.6 mg/dl, C3 690 and C4 was 167 mg/dl), which was so close to present results. The current study values were (588.2±298.5 mg/dl for IgG, 26.44 ± 2.92 mg/dl for IgM, IgA was 6.5±5.5 mg/dl, C3 627.5±221.1 mg/dl and C4 186±71 mg/d)l. Another important study conducted in Turkey published in 2015 by Alkan Ozdemir, Ozer [27] also confirmed what this study found. Their finding regarding IgG, IgM, and their correlation with body weight and gestational age. IgG was 791.5±234.9 mg/dl and IgM 10.6±6.7 mg/dl that seem to be slightly higher to the findings of our study. Strong positive correlation observed between body weight and age with these parameters. The correlation measures of the current study was similar and found that IgG, IgM and C3 protein level were strongly and positively correlated with age and weight with P value <0.05.

To our knowledge, this is the first study conducted in Iraq comparing immunoglobulins and complement factors in hyperbilirubinaemic neonates. No recent published data for the last ten years world widely covered this subject even thorough exploration to the scientific database.

Low level of serum Bilirubin in early fetal life has a strong beneficial effect and it acts as antioxidant, but evolving evidences have observed that at severe hyperbilirubinemia could act as risk factors for disease and injury [28, 29]. Furthermore, Old observation documented in Větvička, Šíma [30] and Jangi, Otterbein [14] showed that low levels of immunoglobulins and complement components were observed in neonates and adults with hyperbilirubinemia and affects their immunological status. The results of present study come in accordance with these findings. An attractive finding of the current study, the positive correlation that observed between age and weight of normal neonates with immune protein factors been lost in high bilirubin level group.

In conclusion, neonatal hyperbilirubinemia associated with an increasing risk of infection and injury that could be due to lower levels of immunoglobulins and complements components in affected neonates. Additionally, the elevation of immune related proteins with age and body weight of jaundiced baby was disturbed.

**Ethical Clearance:** Scientific committee of the Department of Chemistry and Biochemistry, under the rule of College of Medicine, Mustansiriyah University, ethically approves this work.

**Conflicts of Interest:** Researches in present work have no potential conflicts of interest relevant to this article.

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