Evaluation of Serum Sialic Acid and Protein Associated Sialic Acid In Premature Low Birth Weight Neonates

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Abstract:

Objective: In a pioneer study conducted in Iraq, the serum levels of sialic acid and protein associated sialic acid were tested to evaluate their level in premature low birth weight (LBW) neonates The study was conducted in Tikrit Teaching Hospital during the period from the 1st of February 2006 till the 1st of July 2007.

Material & Methods: The study had been done on 300 neonates; they were divided into 4 groups, and these includes 100 apparently healthy normal birth weight mature neonates (NBW), 100 apparently healthy low birth weight premature neonates (LBW), 50 low birth weight premature neonates with birth asphyxia (LBW+BA), and 50 low birth weight premature neonates with respiratory distress syndrome (LBW+RDS).

The following biochemical parameters were measured in sera of the studied neonates and the control groups which included: total sialic acid (TSA) and protein associated sialic acid (PASA).

Results: Revealed that: there is a significant statistical difference between the NBW and the LBW neonates regarding the birth weight and the gestational age while there is no significant difference concerning the age of the newborns. Also there is a significant statistical difference between the LBW neonates and the LBW+BA and LBW+RDS neonates regarding the gestational age, age, and birth weight. The mean serum level of TSA is lower in the LBW in comparison with the NBW. It is also found that the mean value is significantly higher in LBW+BA and LBW+RDS in comparison with LBW and NBW neonates. It is also evident that the mean serum value in LBW+BA is significantly higher than that in LBW+RDS. Serum TSA levels are correlated strongly and positively with serum PASA levels in LBW neonates and LBW+BA and in LBW+RDS .

In conclusion; the mean serum sialic acid and protein associated sialic acid level was found to be significantly higher in LBW+BA and LBW+RDS in comparison with premature LBW and NBW neonates.

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http://doi.org/10.33091/AMJ.0400912011

Introduction

Live born infants delivered before 37 compleleted weeks from the 1st day of the last menstrual period are termed premature by the World Health Organization (WHO). [1]

Infants weighting less than 2500 g at birth is considered as "low birth weight" [2] Many of the infants are small because they are born before their time, others have an abnormally low birth weight for their gestational age. [3]

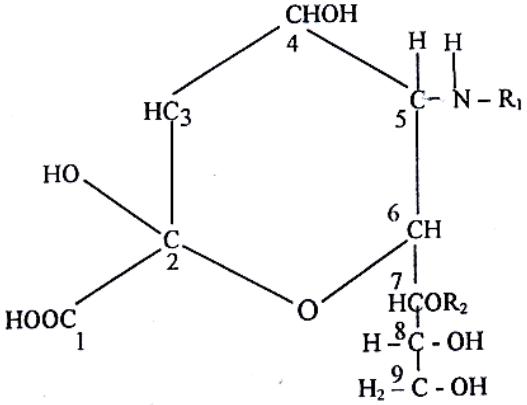
Normal birth weight (NBW) newborn is a newborn with birth weight ranging from 2.5 to 4.6 kg; boys are slightly heavier than girls. [4]

Birth asphyxia (BA) (or hypoxia ischemia encephalopathy) is a multisystem disease results from neonatal asphyxia or ischemia or both that results from different reasons weather it prenatal, natal and postnatal . birth asphyxia affect each organ in the newborns like the brain, heart, lungs , blood , bones , liver and kidneys. [3]

Respiratory distress syndrome (RDS) is a disease that results from immaturity of lungs due to the deficiency in the lung surfactant. It is usually affect preterm newborn although it is affect rarely the mature ones. The deficiency in surfactant leads to decrease in the lung expansion which leads to poor oxygenation of blood lads to ventilation-perfusion mismatch which results in tissue hypoxia. [4]

Sialic Acids (SAs):

"Sialic acids" refer to a family of compounds derived from an substituted nine-carbon chain called neuraminic acid (nine carbon polyhydroxy ketoacid) ($C_9H_{17}O_8N$). [5]



From figure (1) it can be shown that members of SA family of compounds occur in a variety of tissue and body fluids, [6] and in bacteria. [7] The naturally occurring compounds are substituted neuraminic acid derivatives (N-acetyl, N-glycolyl, N, O diacetyl neuraminic acid), these are collectively termed "Sialic acids". [8] The most common form of SA is N-acetylneuraminic acid (NANA).

Neuraminic acid		R_1	R_2
		H	H
Sialic acid	N-acetyl neuraminic acid	O ‖ -C-CH_3	H
	N-glycolyl neuraminic acid	O ‖ -C-CH_2OH	H
	N,O-diacetyl neuraminic acid	O ‖ -C-CH_3	O ‖ -C-CH_3

Sialic acid accounts for a major part of the negative charge on the surface of animal especially mammalian cells, and are important in regulating intercellular contacts and interaction of charged macromolecules with the cell surface. [9]

Sialic acids are found in all tissues and secretion, most are bound carbohydrate moiety of glycoconjugate and glycolipids. They occupied the terminal site in glycoconjugate where they can be released by either mild acid or enzymatic hydrolysis. [9]

Sialic acid is present in the human body in three forms: free sialic acid (FSA), which is present in little amount in serum, protein associated sialic acid (PASA), as a terminal sugar of glycoprotein in the cell membrane and other cell component, and lipid associated sialic acid (LASA; gangliosides), this form represent lipid bound sialic acid which is present in glycolipids.[10]

Sialic acids and other carbohydrate (fucose, glucose, and galactose) are present in plasma membrane gangliosides, other glycolipids, and in the glycoprotein that cover mammalian cell, either as integrated part of plasma membrane element or extending from the outer leaflet called (glycocalyx) which is sometimes of considerable extension.[11,12]

Sialic acids level in serum is thought to be closely related to the stage of some diseases, such as cancer, collagen disease, and inflammatory disease. [13]

Lastly, from the 4th month of life onward, the concentration of serum SA remains approximately the same up into the adult life. [14]

The aim of the study the changes in the serum level of sialic acid and protein associated sialic acid in premature LBW neonates compared with normal birth weight neonates.

Materials and Methods

The study was conducted in Tikrit Teaching Hospital during the period from the 1st of February 2006 till the 1st of July 2007.

Two thauzands newborns of both sexes, with different presentations in Tikrit Teaching Hospital were checked for their age, gestational age, and their birth weights. Only 300 premature LBW neonates were included in this study. Premature neonates included in this study were of comparable ages. Neonates included in this study were collected from: the Neonatal intensive care unit, Delivery room , Operating theater and Out-patient clinic.

Eligibility for inclusion in this study was restricted to newborns with none of the following conditions:

1. Premature infant with normal birth weight. 2. Full term that is small for his gestational age.
3. Overweight infant born at term.
4. Infant of diabetic mother.
5. Infant of thyrotoxic or hypothyroid mother.
6. Infant of hypertensive mother.
7. Syndromes.
8. Those who received oral feeding.

The birth weight of each newborn was measured twice at the same time by using 2 digital baby scales (with subtracting 2 g from the weight of each neonate: the weight of the umbilical clamp) present at the neonatal care unit soon after delivery of the newborn.

Hundred out of the total premature low birth weights were found to be diseased premature newborns; 50 of them with birth asphyxia and 50 with respiratory distress syndrome, their weights were less than 2500 g who were considered as a diseased premature group in this study.

As a part of each newborn medical history, assessment of gestational age by using Dubowitz scoring system, [15] sex of the newborn, age of the newborn, and full physical examination of each newborn to find whether the newborn was healthy or diseased.

Therefore, each newborn included in this study was assessed carefully for the body weight at birth. The assessments of the birth weight were performed by using 2 digital baby scales in order to avoid the instrumental errors. [19]

Three milliliters of umbilical venous blood sample were obtained by using an umbilical catheter from each neonate included in this study soon after the physical examination and before any feeding started. Each blood sample was collected in a plain tube for the estimation of serum TSA, and PASA.

Controls:

Two groups were considered to be as control, the first one composed of 100 apparently healthy NBW, and the second group was also composed of 100 apparently healthy premature LBW neonates.

The same methods and instruments were used in all stages for the apparently healthy control newborns as for the diseased group. A complete history, clinical examination, birth weight, gestational age, and their sex were also considered.

Determination of Serum Total Sialic Acid:

The principle of this method depends on the formation of chromagen by adding resorcinol reagent to the serum in the test tube. The chromagen formed, then was extracted by butyl acetate methanol reagent and measured at 580 nm [16].

Determination of Serum Protein Associated Sialic Acid:

The glycoprotein was precipitated from the serum by addition of ethanol and then dissolved in 0.1 N NaOH [17] and then the developing chromogen is treated accordingly[17]. Protein associated sialic acid was measured in the sera of all studied neonates in order to identify the normal serum level in the low birth weight neonates, and also to compare the results with the results obtained from the normal birth weight neonates.

Results

The results of the present study include the followings:

Physical Characteristics of the Newborns:

The general physical characteristics of the diseased and the apparently healthy neonates included in this study are shown in the table (1). The data obtained shows that there is a significant statistical difference between the NBW and the LBW premature neonates regarding the birth weight and the gestational age at a P value of less than 0.001, while there is no significant difference concerning the age of the newborns. Also there is a significant statistical difference (P value < 0.001) between the LBW neonates and the LBW+BA and LBW+RDS neonates regarding the gestational age, age, and birth weight. The table also reveals that there is no statistical difference between the LBW+BA and the LBW+RDS regarding the gestational age, age, and the birth weight.

Table (1): Physical Characteristics of Newborns.

Groups	No.	Birth weight (g) mean ± SD	Gestational age (wks) mean ± SD	Age (min) mean ± SD
NBW				
Male	**56**	**3176.53 ± 386.60**	**39.26 ± 0.94**	**49.82 ± 26.57**
Female	**44**	**3135.6 ± 411.85**	**39.15 ± 0.98**	**48.40 ± 28.09**
Total	**100**	**3158.65 ± 396.38**	**39.22 ± 0.95**	**49.2 ± 27.12**
LBW				
Male	**49**	**2116.85 ± 206.53**	**34.08 ± 1.81**	**40.10 ± 22.11**
Female	**51**	**2117.82 ± 221.49**	**34.23 ± 2.12**	**37.94 ± 22.71**
Total	**100**	**2117.35 ± 213.21**	**34.16 ± 1.96**	**39.0 ± 22.33**
LBW+BA				
Male	**28**	**1775.53 ± 495.23**	**32.35 ± 3.36**	**54.1 ± 24.03**
Female	**22**	**1802.95 ± 346.70**	**31.9 ± 2.98**	**55.22 ± 26.16**
Total	**50**	**1787.6 ± 432.25**	**32.16 ± 3.03**	**54.6 ± 24.74**
LBW+RDS				
Male	**29**	**1914.27 ± 451.45**	**32.65 ± 3.41**	**63.44 ± 25.84**
Female	**21**	**1966.42 ± 413.99**	**32.85 ± 2.93**	**52.85 ± 24.82**
Total	**50**	**1936.18 ± 432.54**	**32.74 ± 3.19**	**59.0 ± 25.71**

Serum Total Sialic Acid:

Figure (1) illustrates the correlation of TSA in sera of NBW with serum levels of PASA of the same group; this figure reveals also a strong positive correlation (r = +0.97, significant at the 0.01 level) between them.

Serum TSA levels are correlated strongly and positively (r = +0.89, significant at the 0.01 level) with serum PASA levels in LBW neonates, as evident in figure 2.

There is a strong positive correlation (r = +0.31, significant at the 0.01 level) between serum TSA levels and the birth weight in LBW neonates, as in figure 3.

Figure (4) shows a strong positive correlation (r = +0.78, significant at the 0.01
level) between serum TSA levels and serum PASA levels in LBW+BA.

Serum TSA levels are also strongly and positively correlated (r = +0.32, significant at the 0.01 level) with the birth weight in LBW+BA, as shown in figure 5.

Figure (6) shows a strong positive correlation (r = +0.88, significant at the 0.01 level) between serum TSA levels and serum PASA levels in LBW+RDS.

Figure (7) shows a strong positive correlation (r = +0.38, significant at 0.01 level) between serum TSA levels and birth weight in LBW+RDS.

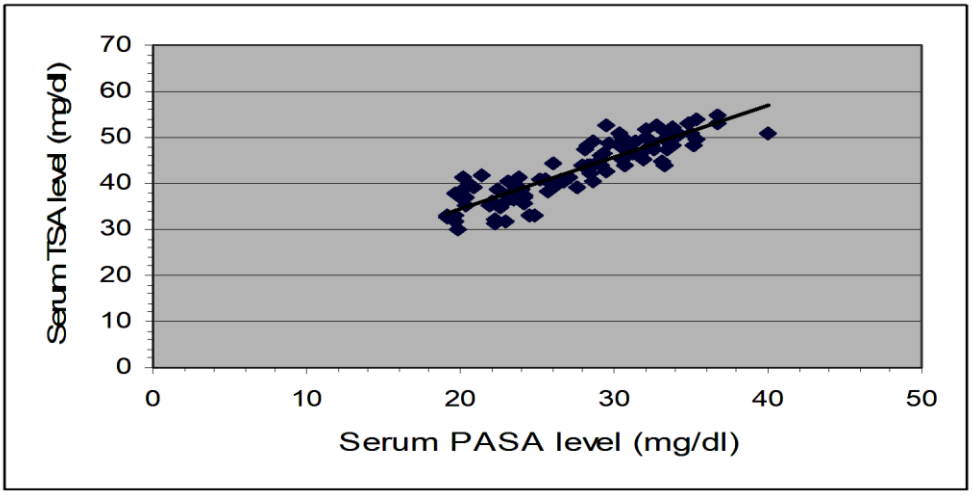


Figure (1): The correlation of serum total sialic acid (TSA) levels to serumprotein associated sialic acid (PASA) levels innormal birth weight (NBW) neonates.

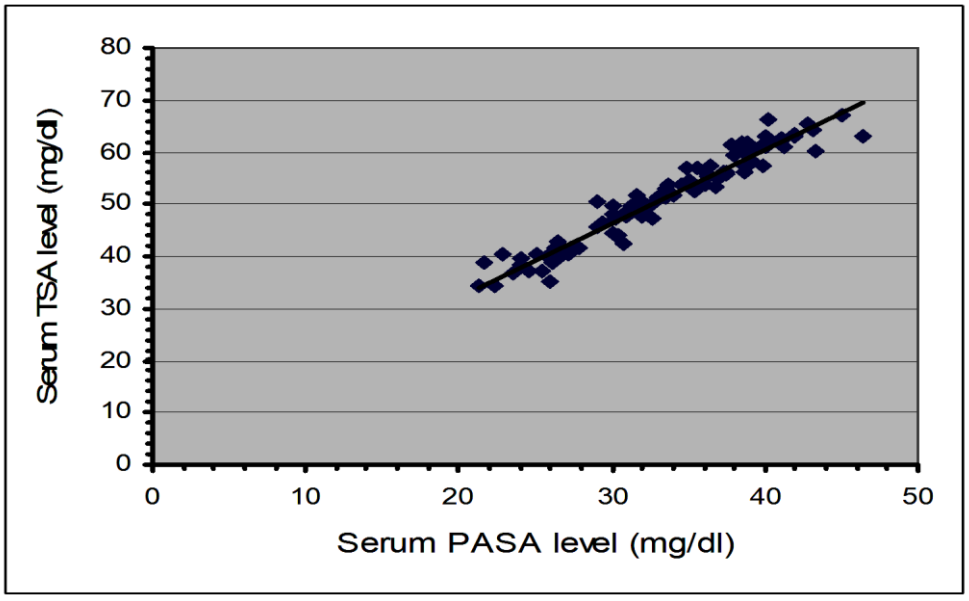


Figure (2): The correlation of serum total sialic acid (TSA) levels to serum protein associated sialic acid (PASA) levels in low birth weight (LBW) neonates.

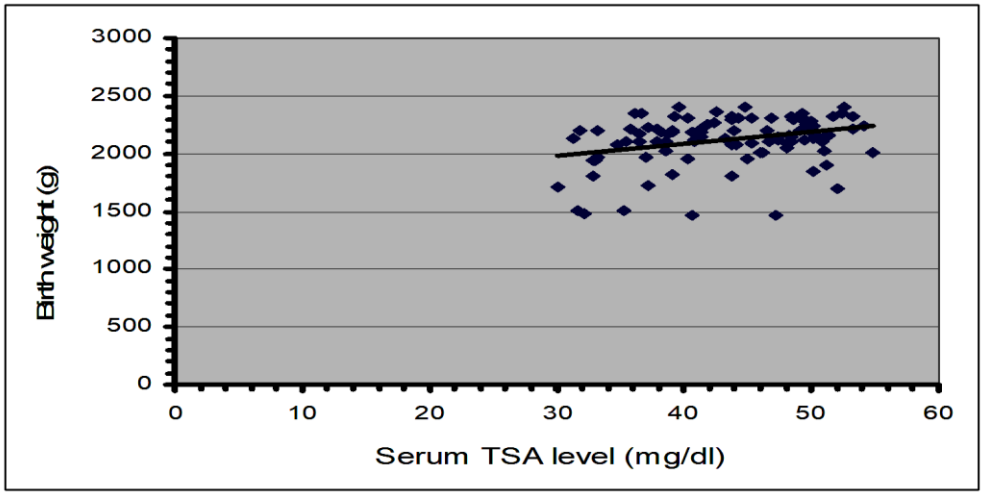


Figure (3): The correlation of serum total sialic acid (TSA) levels to birth weight in low birth weight (LBW) neonates.

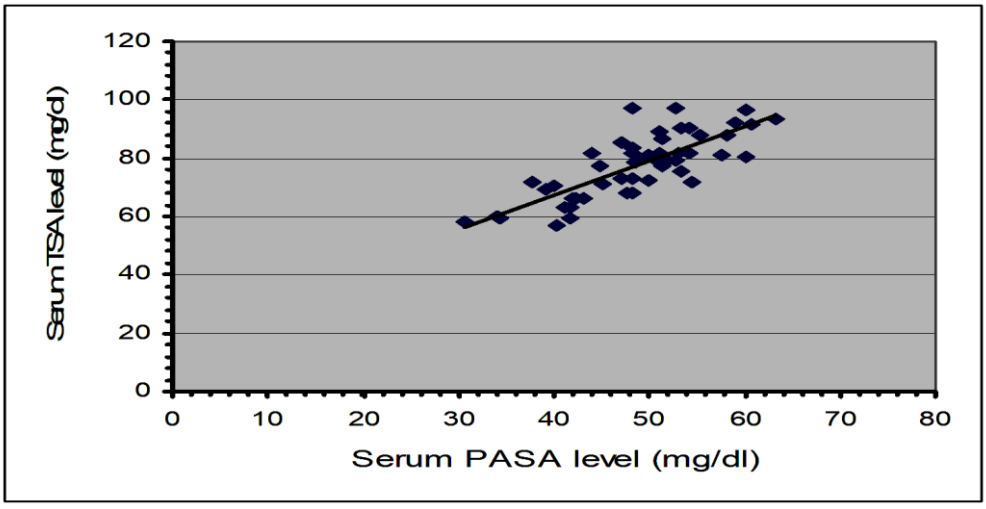


Figure (4): The correlation of serum total sialic acid (TSA) levels to serum protein associated sialic acid (PASA) levels in low birth weight with birth asphyxia (LBW+BA).

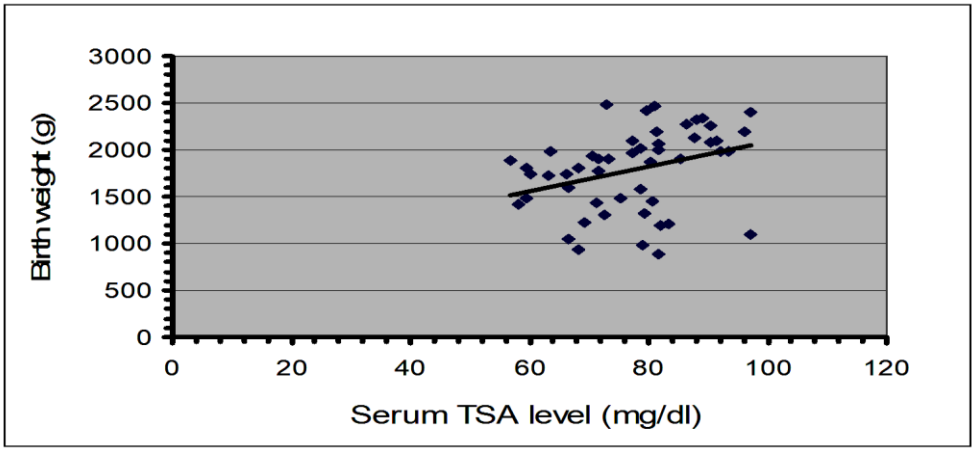


Figure (5): The correlation of serum total sialic acid (TSA) levels to birth weight in low birth weight with birth asphyxia (LBW+BA).

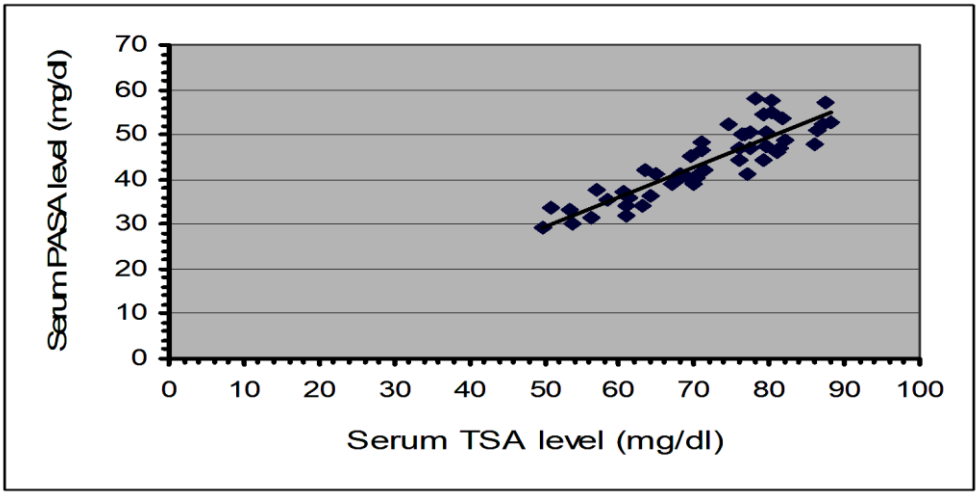


Figure (6): The correlation of serum total sialic acid (TSA) levels to serum protein associated sialic acid (PASA) levels in low birth weight with respiratory distress syndrome (LBW+RDS).

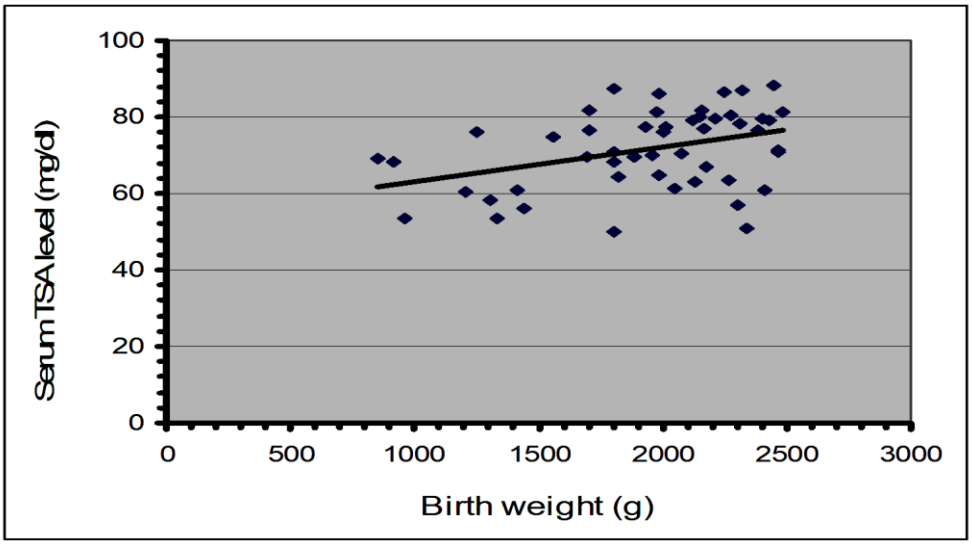


Figure (7): The correlation of serum total sialic acid (TSA) levels to birth weight in low birth weight with respiratory distress syndrome (LBW+RDS).

Serum Protein Associated Sialic Acid:

Figure (8) shows a positive correlation (r = +0.21, significant at the 0.05 level) between serum PASA levels and the birth weight in LBW.

A strong positive correlation (r = +0.37, significant at the 0.01 level) between serum PASA levels and the birth weight in LBW+RDS is shown in figure 9.

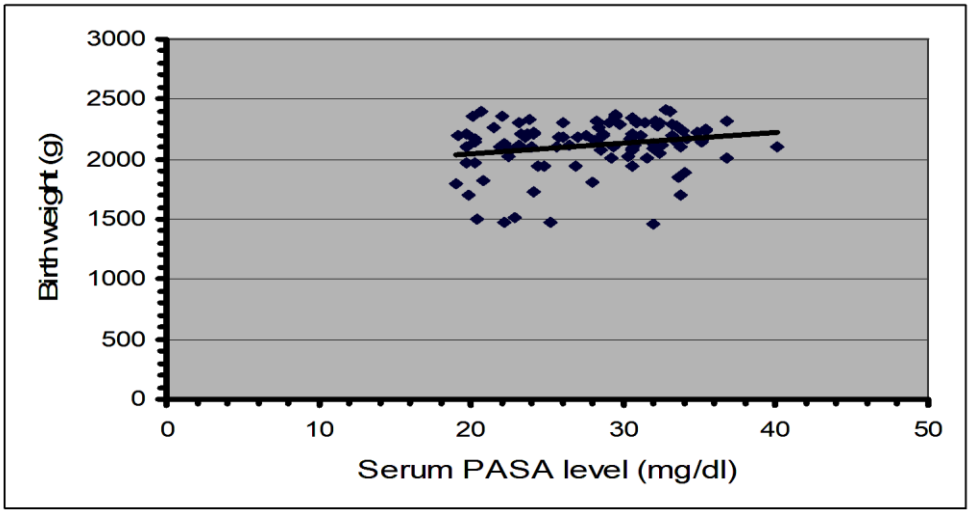


Figure (8): The correlation of serum protein associated sialic acid (PASA) levels to birth weight in low birth weight (LBW) neonates.

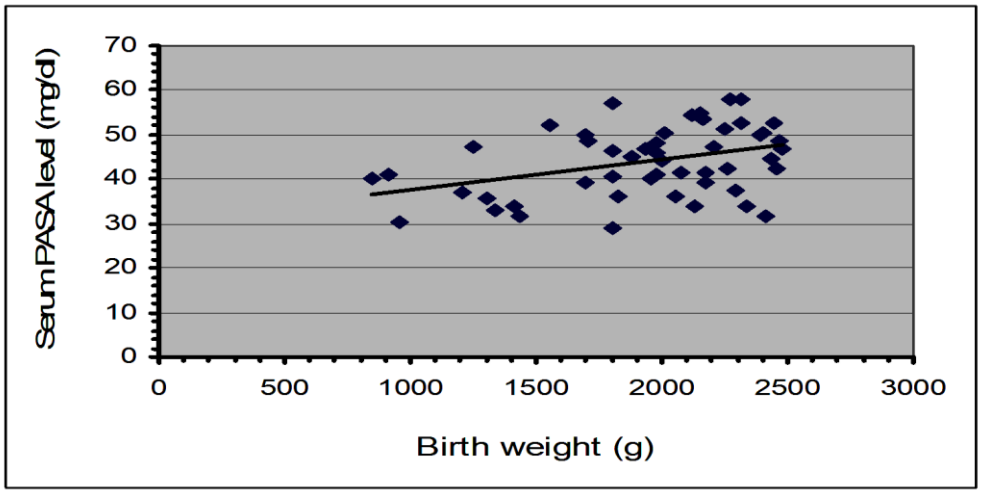


Figure (9): The correlation of serum protein associated sialic acid (PASA) levels to birthweight in low birth weight with respiratory distress syndrome (LBW+RDS).

Discussion

The neonatal period is a highly vulnerable time for an infant, who is completing many of the physiologic and biochemical adjustments required for extrauterine existence. [18]

Physical Characteristics of the Newborns:

The initial examination of a newborn infant should be performed as soon as possible after delivery to detect abnormalities and to establish a baseline for subsequent examination. [18]

In studies on newborns, it is necessary to know the birth weight to decide whether the neonate is a normal or a low birth weight because most of the biochemical parameters differ according to maturity and the birth weight. [19]

Low birth weight neonates are either preterm or small for gestational age newborns. The problems for each group were differ because of the difference in the maturity of organs function and structure.[2]

The data obtained shows that there is a significant statistical difference between the NBW and the LBW neonates regarding the birth weight and the gestational age at a P value of less than 0.001, while there is no significant difference concerning the age of the newborns. This is due to the fact that preterm are those newborns that delivered before 37 week completed weeks while full term is the newborn that born between 38-42 weeks gestation and this explains the difference in the birth weight between the two groups due to the fact that fat deposition is mainly occur at the 3rd trimester so if the baby born prematurely this leads to decrease the birth weight compared with that who born at full gestation.[3]

Also there is a significant statistical difference (P value < 0.001) between the LBW neonates and the LBW+BA and LBW+RDS neonates regarding the gestational age, age, and birth weight. This due the fact that RDS and BA is a disease of premature and extreme prematurity which charachtarised by decreased geastational age and decreased birth weight[2].

The table (1) also reveals that there is no statistical difference between the LBW+BA and the LBW+RDS regarding the gestational age, age, and the birth weight.

The mean serum TSA level in premature LBW is significantly lowers (P < 0.001) than the mean level in NBW neonates. This finding could be attributed to birth weight which is significantly lower in LBW than the NBW neonates; therefore the TSA is lower in LBW neonates. This finding is in agreement with the findings of Wang, *et al* (1998) [20] who considered that sialic acid was a vital component of brain gangliosides which played an essential role in the transmission and storage of information in the brain. These findings explain the findings in this study, since that the fetal and the neonatal periods are the periods of growth for the human brain, therefore serum TSA are expected to be increased with the age, this means that serum TSA levels in NBW are often higher than that in LBW neonates according to this observation.

The finding in this study is not in agreement with the finding by Vural, *et al* (2001) [21] who found an elevation of serum TSA in both mothers and aborted fetuses, but the increase in serum TSA level in their study was secondary to the underlying autoimmune disease.

The mean serum levels of TSA in LBW+BA and LBW+RDS are significantly higher (P < 0.001) than the serum TSA levels in LBW and NBW neonates. This finding is in agreement with the findings of Vural, *et al* (2001) [21] and Wongkhom, *et al* (2001) [22] who found that the determination of TSA yielded diagnostic values for differentiating between diseased and healthy persons.

Abdella, *et al* (2000) [23] found that the serum TSA was a marker for the acute response for some pathological conditions. This finding explains the higher serum levels of TSA in LBW, LBW+BA, and LBW+RDS than the levels in NBW neonates found in the present study.

The finding of a significantly higher (P < 0.01) serum TSA in LBW+BA than that in LBW+RDS; this could be attributed to the stress of hypoxia on tissues in BA is more than that in RDS[24], therefore a greater amount of TSA will be librated to the circulation from the tissues.

The positive correlations of serum TSA levels to serum PASA levels in NBW, LBW, LBW+BA, and LBW+RDS respectively. This finding could be attributed to that; PASA is a part from TSA, therefore an increase in serum TSA is usually associated with an increase in serum PASA level.[25]

In this study, a positive correlation is found between the serum TSA levels and the birth weight in LBW, LBW+BA, and LBW+RDS. This finding may be attributed to the body mass, in which a higher birth weight leads to a higher serum level of TSA.

The mean serum PASA level in LBW neonates is significantly lowers ($P < 0.001$) than that in NBW neonates. This finding could be attributed to birth weight which is significantly lower in LBW than the NBW neonates; therefore the PASA is lower in LBW neonates.

The mean serum PASA levels in LBW+BA and LBW+RDS are significantly higher ($P < 0.001$) than the mean serum levels in NBW and LBW. These findings are in agreement with the findings of Vural, *et al* (2001) [21] and Wongkhom, *et al* (2001). [22]

The mean serum level of PASA in LBW+BA is significantly higher ($P < 0.01$) than the mean serum level in LBW+RDS. This could be attributed to that the stress of hypoxia on tissues in BA is more than that in RDS; [26] therefore a greater amount of PASA will be librated to the circulation from the tissues damage.

There is a positive correlation between serum PASA levels and birth weight in LBW and LBW+RDS, but it is a weak positive correlation in LBW+BA and NBW neonates. This finding may be attributed to the body mass, in which a higher birth weight leads to a higher serum level of PASA.

We Can Conclusion that the serum levels of total serum sialic acid (TSA), protein associated sialic acid (PASA) in low birth weight with birth asphyxia (LBW+BA) and low birth weight with respiratory distress syndrome (LBW+RDS) are significantly higher than the serum levels in normal birth weight (NBW) and low birth weight (LBW) premature neonates.

So We Recommendation the study recommend the following:

1. A further study including premature neonates with normal birth weight to compare their results with those who are low birth weight premature neonates, and full-term neonates who are low birth weight to compare their results with those who are normal birth weight full-term neonates.
2. A serial measurements of serum levels of total sialic acid, and protein associated sialic acid in preterm and full-term neonates to find out when they reaches the adult level.
3. A further study; to measure total sialic acid, and protein associated sialic acid in amniotic fluid in order to assess the fetal distress during pregnancy.
4. A further study; to measure serum total sialic acid, and protein associated sialic acid in monozygotic and dizygotic twins, in order to compare their results with the single live-birth neonates and to see which type of twins undergo more distress during delivery.
5. To measure serum total sialic acid, and protein associated sialic acid in congenital infection, to see if it is of value in assessing the severity of the congenital infections at birth.

References

1. Craig ED, Thompson JMD, and Mitchell EA: Socioeconomic status and preterm birth: New Zealand trends. 1980 to 1999. Arch. Dis. Child Fetal neonatal Ed 2002; 86:F142.
2. Alexander GR, Kogan MD, and Himes JH: Racial differences in birth weight for gestational age and infant mortality in extremely low- risk US populations. Pediatr. Perinat. Epidemiol. 1999; 13:205.
3. Carroli G, Villar J, and Piaggio G: WHO systematic review of randomized controlled trials of routine antenatal care. Lancet. 2001; 357: 1565.
4. Guyer B, Freedman MA, Strobino DM, and Edward P: Annual summary of vital statistics: Trends in the health of

Americans during the 20th century. Pediatrics 2000; 106:1307.
5. O'Brien S: Advance in the biology and treatment of chronic lymphocytic leukemia. Blood 1995; 85: 907.
6. Chatagnon L and Chatagnon P: Clinical bases of sialic acid. Presse. Med. 1955; 63: 1194.
7. Barry GT: Chemistry of sialic acid. Ezpil. J. Med. 1958; 107: 507.
8. Blix FG, Gottschalk A, and Klene E: Compounds related to sialic acid. Nature 1957; 179: 1088.
9. Akinao SM, Hashimato M, and Naovoks MN: Characteristics of sialidase in the rat salivary gland. Enzyme 1989;41: 200-8.
10. Mayes PA: Glycolipids. In: Harper's biochemistry, 21st ed. 1988; Chap. 15:135-6.
11. Schumacher - U, Mukhtar - D, Stehling - P, and Reutter - W. Is the lectin binding pattern of human breast and colon cancer cells influenced by modulators of sialic acid metabolism? Histochem. Cell. Biol. 1996; 106 (6): 599-604.
12. Emmelot P: Biochemical properties of normal and neoplastic cell surface. Rev. J. Cancer. 1973; 9: 319-33.
13. Hakomori S: Changes in serum sialic acid concentration. Annu. Rev. Biochem. 1981; 50: 733-64.
14. Cabezas JA: Serum levels of sialic acid in healthy school age children. Clin. Chem. Acta. 1962; 7: 406-15.
15. Hittner HM, Hirsch NJ, and Rudolph AJ: Assessment of gestational age. J. Pediatr. 1977; 91:455.
16. Svennethalm L: Chromagen extraction in TSA assay. Biochem- Biophys. Acta. 1957; 24:604-11.
17. Katopodis N, Yashar H, and Nancy L: Lipid-associated sialic acid test for the detection of human cancer. Cancer Res. 1982;42: 5270-5.
18. American Academy of Pediatrics, American College of Obstetricia- ns and Gynecologists: Guidelines for Perinatal Care, 4th ed.Elk ed. Elk Grove Village. IL, American Academy of Pediatrics, 1997,p 27.
19. Meites S: Serum cholesterol in newborns. Pediatric Clinical Chemistry, 3rd ed, Washington DC: American Association for Clinical Chemistry 1998; 16-24.
20. Wang B, Miller JB, McMcil Y, and McVeagh P: Sialic acid concent- ration of brain gangliosides: variation among eight mammalian species. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 1998 Jan; 119(1):435-9.
21. Vural P, Akgul C, and Canbaz M: Alterations of total and lipid- bound sialic acid levels in recurrent abortion. Int. J. Fertil. Womens Med. 2001 Nov-Dec; 46(6):315-9.
22. Wongkhom S, Boonla C, Kongkham S, Wongkham C, Bhudhisawasdi V, and Sripa B: Serum total sialic acid in cholangiocarcinoma patients: an ROC curve analysis. Clin. Biochem. 2001 Oct; 34(7):537-41.
23. Abdella N, Akanji AO, Mojiminiyi OA, Al-Assoussi A, and Moussa M: Relation of serum total sialic acid concentration with diabetic complications and cardiovascular risk factors in Kuwaiti Type 2 diabetic patients. Diabetes Res. Clin. Pract. 2000 Sep;50(1):65-72.
24. Moster D, Lie RT, and Irgens LM: The association of Apgar score with subsequent death and cerebral palsy: A population-based study in term infants. J. Pediatr. 2001; 138:798-801.
25. Mayes PA: Glycolipids. In: Harper's biochemistry, 21st ed. 1988; Chap. 15:135-6.
26. American Heart Association and American Academy of Pediatrics. Prematurity . In: Neonatal Resuscitation Textbook, 4th ed, 2000, pp. 208-13.