Neonatal blood rheological parameters at delivery in healthy neonates and in those with morbidities.

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Abstract

Rheological blood parameters of neonates are different form those of adults. Many authors have studied changes in blood rheology in neonates in different clinical disorders. To-date, no one set the normal values for blood rheological parameters in healthy neonates. The aim of this study is to set the norm for rheological blood parameters in healthy newborns and to describe the changes in those parameters in common clinical disorders that affect the newborns. We recruited all the neonates born to mothers experiencing un eventful pregnancies, blood was taken from the umbilical cord right after the delivery. In this time period we recruited 4985 neonate. From this huge database we were able to set the standards for blood rheology in neonates, namely plasma viscosity of 1.06 +/-0.072 mPa, erythrocyte aggregation at stasis of 2.41 +/- 2.74 s^{-1} and erythrocyte aggregation under low shear forces of 8.51 +/- 6.38 s^{-1}. These values changed significantly in some diseased neonates. This is the largest study investigating normal rheological parameters and deviations from the norm in common clinical disorders occurring in this early stage of life.

Keywords. Neonate, blood rheology, SGA, LBW.

Introduction

Rheological blood properties of newborns are different from those of adults [1, 2]. Fetal and neonatal rheological blood parameters were studied in healthy neonates and in different neonatal disorders as well and the changes in these parameters were traced in those studies [3, 4 and 5]. However, those studies included in the most, a small number of neonates and thus could not provide enough statistical power to generalize their findings. Second, no study was performed to date to define the norm of rheological parameters in newborns. This when achieved, will not only provide a reference of normality, but also will be of great help to study neonatal rheological parameters and their changes in different clinical situation by simply comparing them to the norm. Moreover, there are many disorders in this very early stage of life where blood rheology might help explaining normal physiological findings or provide helpful clues for a better understanding of mechanisms of disease. Among the few studies investigating rheological parameters and their changes in association with clinical disorders in neonates Mandelbaum and his coworkers found a correlation between plasma viscosity on one hand and cardiac output and vasodilatation on the other hand when following neonates in the first five postnatal days. Plasma viscosity played a central role in those dynamic changes that he validated. That is why we believe studying blood rheology particularly in neonates will help understanding disease mechanisms, or at least provide information that will help us better understand those mechanisms [6].

In the current study, our aim was to define the norm for different rheological blood parameters in healthy neonates born after uneventful pregnancies and through uncomplicated deliveries with normal birth weight and to compare them to the rheological findings registered in neonates with common clinical disorders.

Patients and methods

All neonates, who completed 24 weeks' gestation who were delivered in the time period from January 1990 to the end of December 1996 were principally eligible to be consecutively included into this retrospective investigation, regardless of the birth weight. The gender and birth weight were registered for every neonate, besides, an umbilical cord blood sample was taken directly after the delivery for rheological examination, hemoglobin concentration, hematocrit, blood sugar level analysis and umbilical cord pH. We did not only study the rheological parameters in healthy neonates, but we also studied them in neonates with low birth weight (LBW) whose birth weight is lower than 2600 gm and small for gestational age (SGA) neonates whose birth weight is lower than the 25^{th} percentile adjusted for gestational age at birth, and stratified this group into SGA lower than 25^{th} percentile but more than the 10^{th} percentile, SGA lower than 10^{th} percentile but more than the 5^{th} percentile and SGA lower than the 5^{th} percentile. We also included neonates that were preterm at birth, namely those who were delivered before completed 36 weeks of gestation and after complete 24 weeks' gestation. In addition to tabulating and calculating the mean values for rheological parameters in healthy and morbid neonates, we analysed the collected rheological data for significant variations caused by all those studied factors.

Rheological parameters:

Estimations of blood rheological and other studied parameters were performed after delivery of the baby and directly after cutting the umbilical cord. After minimal stasis of the blood in a small segment of the clamped and already transected umbilical cord at the maternal side, blood was drawn from the umbilical vein using a 20 gauge needle supplied with a vacuum tube. Blood was collected in vacuum tubes containing 1:10

potassium EDTA (ethylene diamine tetraacetic acid) and rheological estimations were immediately performed in the laboratory of the Department of Gynecology & Obstetrics according to ICSH guidelines (International Committee for Standardization in Haematology) [7]. Red Blood Cell aggregation (RBC aggregation) was estimated using a photometric rheoscope developed by Schmid- Schoenbein et al [8].. For determination of plasma viscosity vacuum tubes were centrifuged for 20 minutes (2000g at 4◦C) whereas probes from the middle-layer of the plasma were obtained and inserted into and measured with the system of a Capillary tube viscosimeter (KSPV 1 Fresenius, Bad Homburg Germany) at 37◦C according to Jung et al [9]. (normal adult range: 1.14– 1.34 m Pa). A detailed description of the steps of the various rheological tests performed is cited elsewhere, where we performed our tests exactly as cited [10].

Statistical analysis:

Descriptive analysis included mean values ± standard deviations, median, inter quartile range. Differences between groups were assessed using the one-way analysis of variation (ANOVA) test. Two sided Pearson's correlation coefficient was used to correlate different parameters. p values of less than 0.05 were considered statistically significant. All tests are performed with assuming a confidence interval of 95%. Statistical analyses were conducted using PSPP-project version 0.7.9, released February 2012.

Results

In this retrospective cross sectional study and during the aformentioned study time period we collected data from 4985 neonates right after delivery. As stated before, we studied some important clinical problems in those neonates, and compared their findings to those of the healthy neonates that we included also in our cohort.

1 Cohort characteristics

Table 1 shows the frequency distribution of healthy neonates in addition to neonatal morbidities studied in our cohort.

2 Rheological parameters of our cohort population in absence of morbidities

In order to set the normal values of the different rheological parameters, we had to collect these values from all the healthy neonates included in our cohort, tabulate and analyse them to come out with the targeted values. These are presented in table 2, for healthy neonates, male and female neonates as well.

From this table plasma viscosity of 1.06 +/-0.072 mPa, erythrocyte aggregation at stasis of 2.41 +/- 2.74 s^{-1} and erythrocyte aggregation under low shear forces of 8.51 +/- 6.38 s^{-1} could be considered as the normal value for a healthy full term neonate, after an uneventful pregnancy with normal birth weight and no apparent disease. These values are not statistically significant different between male and female neonates except for erythrocyte aggregation at stasis (2.31 +/- 2.62) where the mean values of female neonates are weakly statistically significant lower than the means for male neonates (2.47 +/- 2.81) $p = 0.041$.

Rheological parameters in healthy newborn babies with their birth weight between 25^{th} and 75^{th} percentiles are graphically represented in the histogram in **Figure 1** showing

the frequency distribution of plasma viscosity, erythrocyte aggregation at stasis and under low shear forces in this group of neonates.

This figure shows the normal shaped Gaussian frequency distribution curve of both the plasma viscosity and erythrocyte aggregation under low shear forces, where the erythrocyte aggregation under low shear forces show some left hand shift of the curve, most probably because the median (7.6 s^{-1}) of the observations lies slightly to the left of the mean (8.51 s^{-1}). The curve appears however somehow different when analyzing erythrocyte aggregation at stasis, whereas approximately one third of the values registered are slightly above 0.0, where 21% of the values are 0.1 s^{-1} and 16% read 0.2 s^{-1} with a median of 1.4 s^{-1} that obviously lies to the left of the mean 2.41 s^{-1}. The SD of erythrocyte aggregation at stasis (2.74 s^{-1}) is also more than the mean, which explains the non-peaked shape of the frequency distribution curve and its extension over a wide area.

3.Rheological parameters in different clinical disorders studied in the neonates in our cohort.

The different neonatal blood rheological parameters in the different clinical situations we studied were analysed for statistically significant variations and presented in table 3.

Presence of morbidities in general was accompanied with statistically significant differences between the means of the values of plasma viscosity and erythrocyte aggregation at stasis. The mean values of Erythrocyte aggregation under low shear forces however were not statistically significant different from the mean values of healthy neonates. This is clearly graphically represented in **Figures 2, 3 and 4**, where one could see the obviously lower mean value of the plasma viscosity in the morbid neonates group (1.04 mPa) when compared to the healthy ones(1.06 mPa). The same

can also be noted in erythrocyte aggregation at stasis box plot; the mean value of morbid neonates is 2.2 s^{-1} and 2.41 s^{-1} for healthy ones.

Some rheological parameters in preterm neonates were statistically significant different from those in healthy term neonates. Plasma viscosity (1.02 mPa) and erythrocyte aggregation at stasis (1.98 s^{-1}) were statistically significant lower than the registered normal values for healthy term newborns (1.06 mPa and 2.41 s^{-1} respectively). Variations in erythrocyte aggregation under low shear forces did not show statistically significant differences between preterm and term neonates.

LBW neonates showed a statistically significant lower mean value for plasma viscosity (1.03 mPa) when compared to neonates with normal birth weights (1.06 mPa). Other rheological parameters were however not statistically significant different from the means of neonates with normal birth weights.

SGA neonates did not generally show statistically significant different means of rheological blood parameters from healthy neonates with normal birth weight. Erythrocyte aggregation under low shear forces in the group with SGA < 10^{th} percentile and > 5^{th} percentile (7.89 s^{-1}) was however statistically significant lower than when compared to neonates with normal birth weight (8.51 s^{-1}) $p = 0.034$.

4 Rheological blood parameters in different pH values

Due to the clinical importance of umbilical cord blood pH right after the delivery we paid special attention to this entity. Neonates were categorized according to the umbilical cord pH value into three different groups; pH > 7.2, 7.2 > pH > 7.0, and pH < 7.0. The variation of the means of rheological blood parameters in these three groups are represented in table 4.

This table presents the values of the studied rheological blood parameters namely plasma viscosity, erythrocyte aggregation at stasis and erythrocyte aggregation under low shear forces in neonates with normal pH, light acidotic and severe acidotic umbilical cord pH. The plasma viscosity in the neonates in the light acidotic group (7.2 > pH > 7.0) was statistically significant higher (1.07 mPa) than the plasma viscosity in the group with normal pH values (1.06 mPa) $p = 0.027$. Otherwise the means of the various rheological blood parameters in both acidotic umbilical blood pH groups were not statistically significant different from those neonates with normal umbilical cord pH values.

Discussion

We claim through this study to be the first study group that sets the norm for rheological blood parameters in healthy neonates, namely; plasma viscosity, erythrocyte aggregation at stasis and under low shear forces. We achieved this aim through recruiting a big number of neonates over a relatively long time period, with which we also claim to be the biggest rheological study done on neonates to date. A plasma viscosity of 1.06 +/-0.072 mPa, erythrocyte aggregation at stasis of 2.41 +/- 2.74 s^{-1} and erythrocyte aggregation under low shear forces of 8.51 +/- 6.38 s^{-1} could be considered as the normal value for a healthy neonate with normal birth weight and normal umbilical cord pH at birth.

In addition to setting the normal values for blood rheological parameters in healthy neonates, we also studied rheological parameters in many clinical disorders. Many authors studied rheological blood parameters in neonates in normal and disease states [3 – 6, 11-13] but no one studied this large number of neonates which gives this work a good credibility due to the statistical power of the results. In this study we analysed the rheological blood parameters in neonates which were SGA, LBW, neonates with acidotic umbilical cord pH right after delivery and preterm neonates. Our analysis revealed a weak significance of the difference between the values of erythrocyte aggregation under low shear forces in one subgroup of the SGA neonates whose birth weight is < 10^{th} percentile for gestational age but > 5^{th} birth weight percentile. We did an online literature search at pubmed.org with the keywords (SGA, Blood rheology, neonate and erythrocyte aggregation) but found no results matching SGA and blood rheology. This finding might hypothetically be due to the fact that some SGA neonates are healthy babies but are just constitutionally predestined to be small newborns small.

One point in favor of this hypothesis, is the absence of any significant difference between the rheological values of SGA neonates and those with normal birth weight. Unfortunately, we do not have enough data in the literature to confirm this finding lest explain it. This point needs to be further investigated and explained.

The same situation applies to the weak significant difference in mean values of erythrocyte aggregation at stasis between male and female neonates. This was not reported any where else in the literature according to our literature search. The only work that tackled gender differences in neonatal morbidity was presented by Stark and his co-workers who found out that significantly more blood flows in the peripheral circulation of male preterm neonates than female counterparts and that the preterm male neonates show more vasodilational response to stimuli the more the basal flow rate they have [11]. This could not however help us better understand and explain our finding that the erythrocyte aggregation at stasis is significantly higher in normal newborn males than in their female matches. This finding has to be further confirmed and scrutinized in future work.

Our data showed also that LBW neonates have significantly lower plasma viscosity when compared to those with normal birth weight. Plasma viscosity is proportionate to plasma protein concentration [4, 6], and the possibility that the LBW might have been due to preterm birth or growth restriction, such disease entities which affect liver production of plasma proteins might explain this lower plasma viscosity. This point however needs to be thoroughly studied in a separate more detailed study.

The plasma viscosity and erythrocyte aggregation at stasis were also found to be significantly lower in morbid neonates in comparison to their values in healthy ones. The significant difference in plasma viscosity might exist for exactly the same reason as

it is with LBW neonates, namely the not yet well developed liver functions at this early time in life, especially if the newborn is a LBW due to growth retardation where it already suffers depleted liver reserves or it is a preterm neonate were the liver functions are still not well developed and hence less ability to function and produce plasma proteins. Lower erythrocyte aggregation at stasis, however weakly significant, can also be explained due to the same reason as the plasma viscosity, as erythrocyte aggregation is affected with plasma protein blood levels [5]. The significant difference in the morbid neonates group can also be explained by the fact that both LBW and preterm neonates are included in this group, and both neonates have significantly lower plasma viscosity than normal neonates, and this might only be the impact of including those newborn in the same group with other newborns suffering other clinical disorders. This however does not affect the authenticity and the statistical power of the analysis, because the morbid neonates are sub-grouped and analysed separately.

The significantly lower plasma viscosity and erythrocyte aggregation at stasis in preterm neonates compared to term neonates could be explained also by the same reason as with LBW neonates. However, being one of the most common neonatal killers, this finding need to be thoroughly analysed and intensively studied to help explain and understand this finding. This particular finding and due to its utmost importance is going to be the scope of a further work from our study group.

The weakly significant higher plasma viscosity (1.07 mPa) in the light acidotic pH group ($7.2 > pH > 7.0$) when compared to the normal pH group (Plasma viscosity 1.06 mPa) was the only significant change noted in rheological blood parameters in relation to changes in blood pH. While trying to understand , explain and correlate the increase in plasma viscosity in light acidotic newly born infants that we found, we could not find

any literature tackling this observation. The only interesting studies studying changes in rheological parameters in response to changes in blood pH were performed on adults especially athelets, or in a general context of exercise, but was never performed on neonates (Pubmed.org literature search). Varlet-Marie and co-workers found a significant positive correlation between erythrocyte aggregation and lactate accumulation, and hence decreasing pH in the circulation during exercise, they also observed a significant increase in plasma viscosity as a result of increasing lactate when the athelete is on the edge of overtraining syndrome (i.e. acidotic pH values) [12]. In a further trial to explain the hemorheological changes in response to exercise and changing the hematological milieu, Elsayed and his colleagues related, however, his observation of an increase in plasma viscosity after vigorous exercise to hemoconcentration and not to changes in blood lactate levels [13] in contradiction to Varlet-Marie et al. who related the plasma viscosity in crease to increasing blood lactate levels but could not explain a reasonable mechanism for this observation. Romain et al. could not prove, however, through their meta-analysis the findings of the above mentioned authors. They found heterogenous data correlating plasma viscosity and exercise and hence blood pH and lactate concentrations and they could not find a significant correlation between changes in plasma viscosity and pH changes or lactate levels during exercise in adults [14]. Ahmadizad et al. found significant but temporary increase in plasma viscosity and erythrocyte aggregation in response to acute exercise, but they could not explain why and how this happens [15]. We tried through this literature search to find an explanation for this increase in plasma viscosity in our cohort of neonates, but unfortunately this effort was unfruitful. Our hypothesis explaining this observation is the physiologic effect of blood pH on the plasma proteins making some

of them more liable to clump together and hence increase the viscosity, but this would have also probably lead to increase in erythrocyte aggregation, which is not observed in our study. Therefore we believe more work should be designed to investigate this observation.

One of the drawbacks of our study is the obvious overlap between the different morbidity groups which could have a negative impact on the statistical analysis. This could be clearly demonstrated in table 1 where the sum of the percentages of all the groups is more than 100%. This happened because one newborn could be included in two groups simultaneously, for example, the LBW group, in the SGA group and in the preterm group in the same time. This is, however, inevitable when the newborn meets the criteria for the three groups in the same time, and we tried to avoid its impact on the authenticity of our results by doing all our calculations with a 95% confidence interval.

To conclude, this study provides for the first time the normal values of rheological blood parameters in healthy newborns and in the same time traces the main changes in blood rheology in neonates with common morbidities in this early stage of life with good statistical power due to the large number of included healthy and diseased neonates.

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Tables

Morbidity studied		Frequency n (%)
SGA	**<5th percentile**	138 (2.8)
	<10th, > 5th percentile	124 (2.5)
	<25th, >10th percentile	377 (7.6)
	Total	639 (12.8)
LBW		460 (9.2)
Preterm		465 (9.3)
Healthy neonates with normal birth weight		3925 (79.1)

Table 1. A frequency distribution table showing the different morbidities detected in our cohort. Small for Gestational Age (SGA) is defined as lower than the 25th percentile adjusted for gestational age at delivery, and the neonates are stratified as <25th and more than the 10th percentile, <10th and more than the 5th percentile and <5th percentile. Preterm is defined as lower than completed 36 weeks of gestation, low birth weight (LBW) is defined as lower as 2600 gm birth weight.

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		Plasma viscosity	Erythrocyte aggregation at stasis	Erythrocyte aggregation under low shear forces	Number
Healthy neonate with normal birth weight	**Mean +/- SD**	1.06 +/-0.072	2.41 +/- 2.74	8.51 +/- 6.38	3925
	Median	1.06	1.4	7.6	
	Range	0.9 – 1.23	0.1 – 26.5	0.2 – 99.9	
Female neonate	**Mean +/- SD**	1.06 +/- 0.71	2.31 +/- 2.62	8.39 +/- 6.79	2373
	Median	1.06	1.3	7.5	
	Range	0.9 – 1.23	0.1 – 26.5	0.2 – 99.9	
Male neonate	**Mean +/- SD**	1.05 +/- 0.08	2.47 +/- 2.81	8.49 +/- 5.91	2509
	Median	1.05	1.4	7.6	
	Range	0.03 – 1.23	0.1 – 27.5	0.1 – 99.9	
	p-value	0.123	**0.041***	0.596	

367 Table 2. Frequency distribution table of the rheological parameters of healthy neonates in addition to male and female
368 neonates in our cohort. p-values refer to the ANOVA test when comparing the means of male and female neonates to
369 each other. $p < 0.05$ is a statistically significant value.

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		Plasma viscosity	Erythrocyte aggregation at stasis	Erythrocyte aggregation under low shear forces	Number
Morbidity exists	**Mean +/- SD**	1.04 +/- 0.08	2.2 +/- 2.64	8.36 +/- 7.17	961
	Median	1.06	1.4	7.6	
	Range	0.05 – 1.23	0.1 – 27.4	0.1 – 99.9	
	p-value	**<0.0001***	**0.015***	0.651	
SGA (collectively)	**Mean +/- SD**	1.05 +/- 0.079	1.2 +/- 2.57	6.9 +/- 6.17	621
	Median	1.05	1.2	6.9	
	Range	0.03 – 1.23	0.1 – 16.2	0.2 – 99.9	
	p-value	0.158	0.204	0.069	
SGA <25th but >10th percentile	**Mean +/- SD**	1.05 +/- 0.07	2.18 +/- 2.39	7.85 +/- 6.63	370
	Median	1.05	1.2	6.9	
	Range	0.84 – 1.23	0.1 – 12.9	0.4 – 99.9	
	p-value	0.557	0.124	0.058	
SGA <10th but >5th percentile	**Mean +/- SD**	1.05 +/- 0.12	2.14 +/- 2.39	7.89 +/- 5.42	121
	Median	1.06	1.3	6.55	
	Range	0.03 – 1.18	0.1 – 11.7	0.2 – 29.2	
	p-value	0.287	0.067	**0.034***	
SGA <5th percentile	**Mean +/- SD**	1.05 +/- 0.07	2.6 +/- 3.16	8.58 +/- 5.41	130
	Median	1.05	1.1	7.4	
	Range	0.82-1.19	0.1-16.2	0.7-26	
	p-value	0.158	0.204	0.069	
LBW	**Mean +/- SD**	1.03+/-0.07	2.16+/-2.83	8.38+/-6.94	433

	Median	1.03	1	7.2	
	Range	0.79-1.23	0.1-19.2	0.2-99.9	
	p-value	**< 0.0001***	0.069	0.849	
Preterm neonate	**Mean +/- SD**	1.02+/-0.07	1.98+/-2.69	8.66+/-8.07	439
	Median	1.01	0.9	7.6	
	Range	0.5-1.19	0.1-19.2	0.2-99.9	
	p-value	**< 0.0001***	**0.001***	0.462	

Table 3. Frequency distribution table of the rheological parameters of different clinical neonatal disorders divided into subgroups. p-values refer to the ANOVA test when comparing the means of the corresponding groups of neonates to the mean of normal healthy neonates. $p < 0.05$ is a statistically significant value. The term morbidities refers to any abnormal clinical situation which is mutually exclusive i.e. each neonate is counted only once, either as SGA or as LBW or as preterm. All values are calculated at a CI =/> 95%.

		Plasma viscosity	Erythrocyte aggregation at stasis	Erythrocyte aggregation under low shear forces
pH > 7.2	**N**	4671	4680	4653
	Mean +/- SD	1.06 +/- 0.074	2.4 +/- 2.71	8.3 +/- 5.16
	Range	0.03 – 1.23	0.1 – 27.4	0.1 51.2
7.2 > pH > 7.0	**N**	176	178	175
	Mean +/- SD	1.07 +/- 0.07	2.26 + /- 2.67	7.7 +/- 4.9
	Range	0.91 – 1.22	0.1 – 21.8	0.7 – 40.7
	T-test	-2.211	0.609	1.498
	p	**0.027***	0.542	0.134
pH < 7.0	**N**	11	11	11
	Mean +/- SD	1.05 +/- 0.07	2.57 +/- 2.74	10.42 +/- 8.01
	Range	0.93 – 1.17	0.1 – 6.3	3.3 – 29.2
	T-test	0.386	-0.224	-1.358
	p	0.699	0.823	0.175

Table 4. T-test for analyzing the variation in the means of values of rheological parameters in groups with different umbilical cord pH values. A statistically significant p value is = or < 0.05. * denotes a statistically significant correlation. All values are calculated at a CI =/> 95%.

Figures and captions

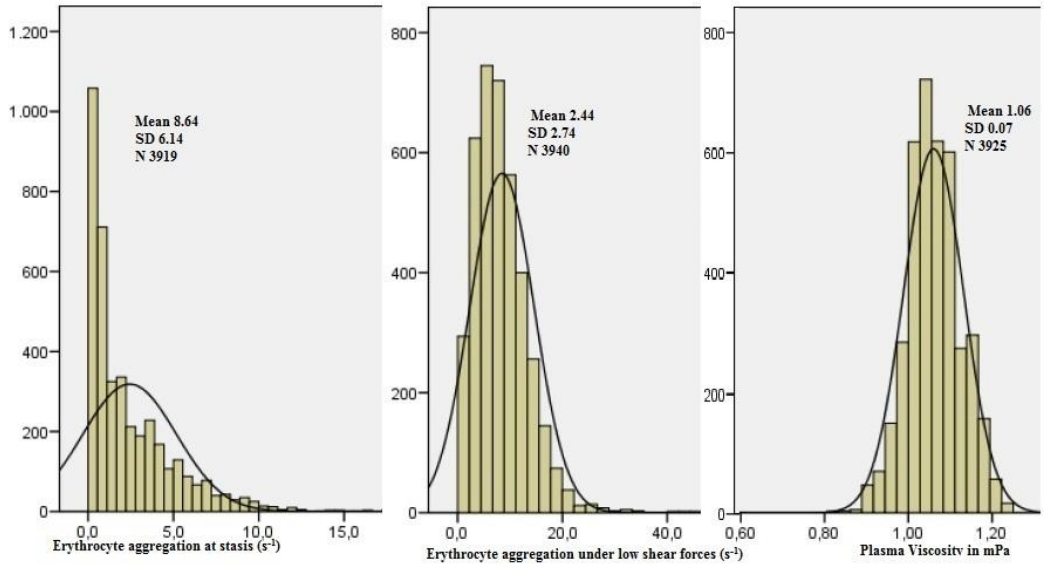

Figure 1. Histogram of frequency distribution of plasma viscosity, erythrocyte aggregation both at stasis and under low shear forces in newborns with birth weight between the 25th and the 75th percentile.

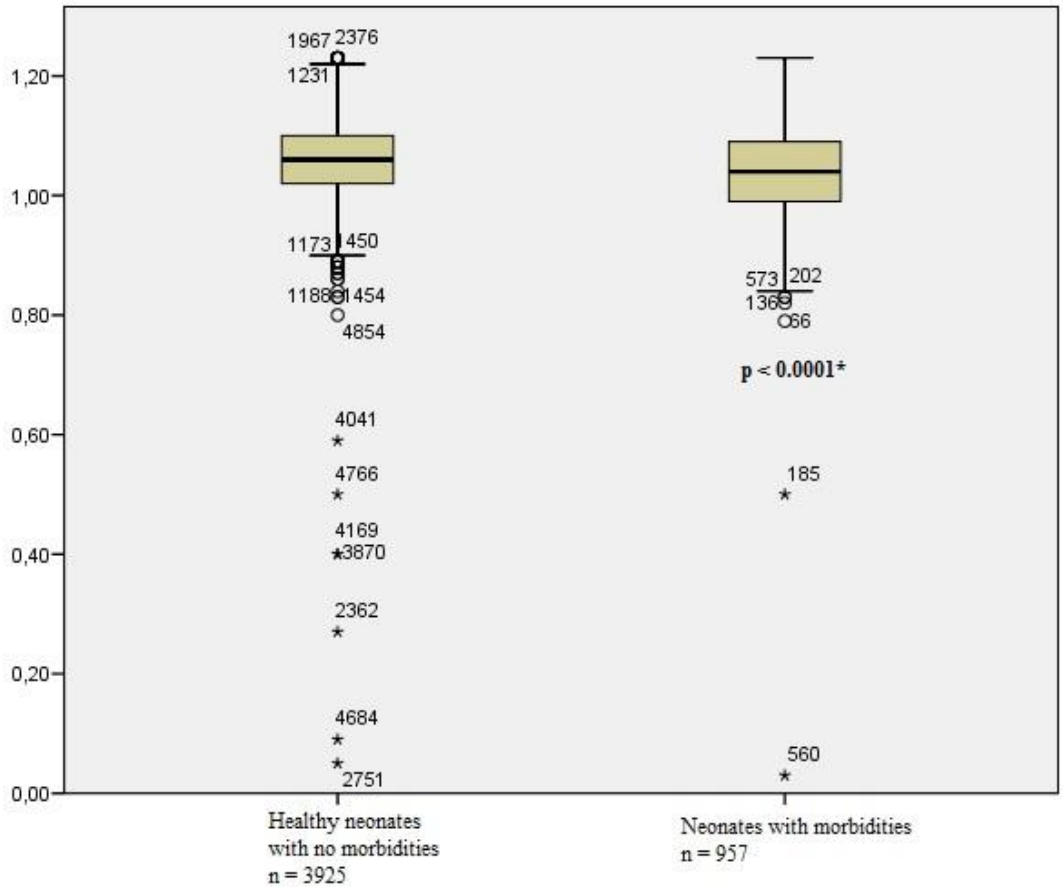

Figure 2. A box plot showing plasma viscosity in healthy neonates and those with morbidities. (Median, 25 to 75% interquartiles, minimum and maximum values, outliers). A statistically significant p value is = or < 0.05. * denotes a statistically significant correlation. All values are calculated at a CI =/> 95%.

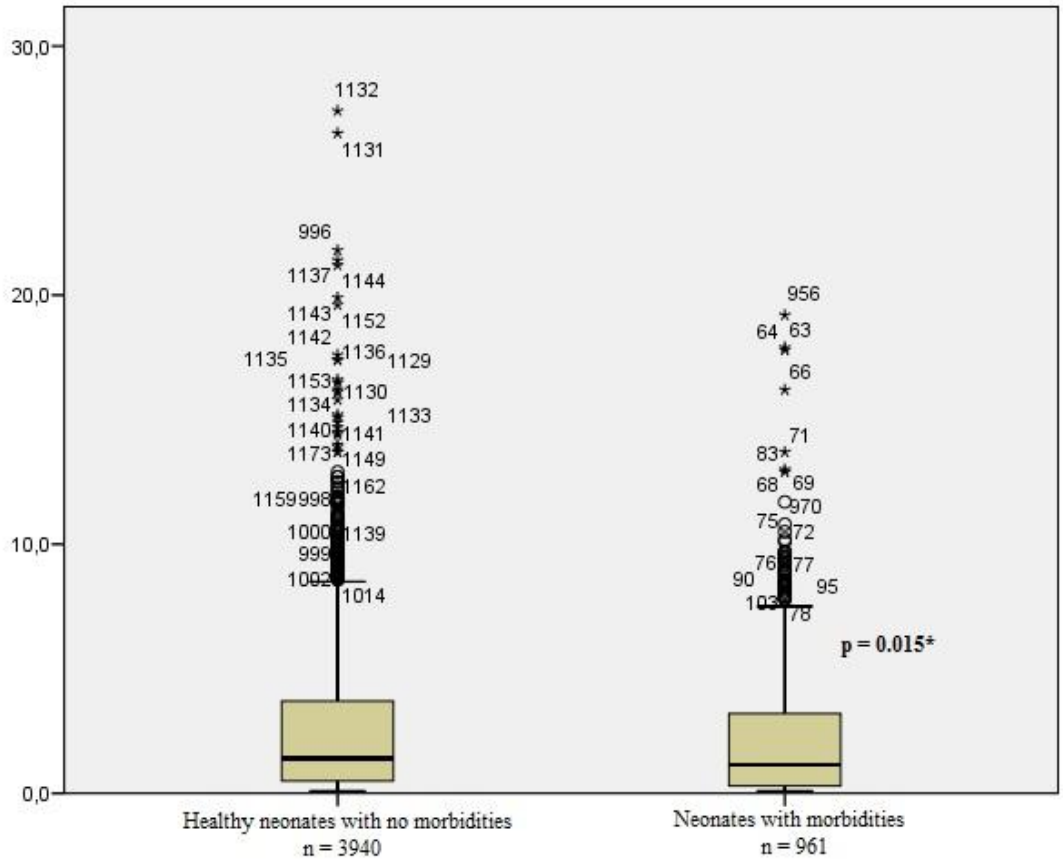

Figure 3. A box plot showing erythrocyte aggregation at stasis in healthy neonates and those with morbidities. (Median, 25 to 75% interquartiles, minimum and maximum values, outliers). A statistically significant p value is = or < 0.05. * denotes a statistically significant correlation. All values are calculated at a CI =/> 95%.

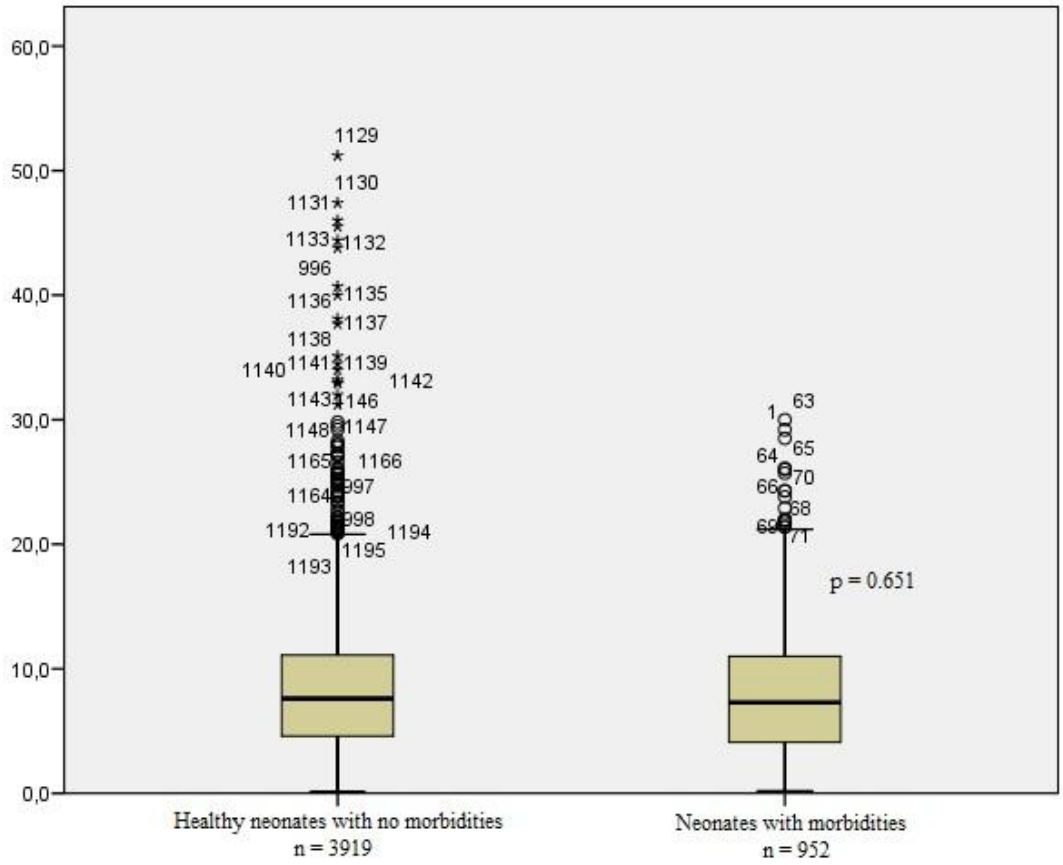

Figure 4. A box plot showing erythrocyte aggregation under low shear forces in healthy neonates and those with morbidities. (Median, 25 to 75% interquartiles, minimum and maximum values, outliers). A statistically significant p value is = or < 0.05. * denotes a statistically significant correlation. All values are calculated at a CI =/> 95%.