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CUPRINS

UNDERSTANDING THE BLOOD-BRAIN BARRIER

CRISTIAN SCHEAU, ANDREEA ELENA SCHEAU, RALUCA IOANA PAPACOCEA, CĂTĂLINA MARIANA CIORNEI, IOANA ANCA BĂDĂRĂU
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ABSTRACT
The blood-brain barrier is a mostly impermeable structure separating the blood from the central nervous system. Its existence has been deduced by scientists in the 19th century, but technological progress allowed viewing the constituency of the blood-brain barrier only in the mid-20th century. The study of the blood-brain barrier continues even today, because all the roles of its components are not fully understood, as new information is uncovered by exploring it in both physiological and pathological situations, in-vivo and in-vitro. The endothelial cells with their tight junctions, the basal lamina, the astrocyte feet, the pericytes and other structural parts of the blood-brain barrier all contribute towards its complex degree of impermeability due to mechanical, electrical, chemical and immune factors.
Keywords: blood-brain barrier, ultrastructure, neurophysiology

INTRODUCTION

The Blood-Brain Barrier (BBB) is an important structure which functions as an interface between the blood and the central nervous system, restricting the diffusion of most blood compounds towards the brain, but facilitating complex signaling and regulation mechanisms involved in the body's homeostasis. It has a complex morphology with different types of constituents, building towards intensively studied but not yet completely understood selective gateway between the central nervous system and the circulating blood.

THE FORETELLING OF THE BLOOD-BRAIN BARRIER

The first observations that indirectly pointed towards the existence of a barrier between the blood and the central nervous system belong to Paul Enrlich, which in 1885 noticed that with the injection of a dye in the peripheral venous system of an animal, all the organs were stained except the brain and spinal cord [1]. He initially attributed this particular finding to the difference in binding affinities of the dye.

The hinting towards the presence of an actual barrier was done by Max Lewandowsky in 1900, when he noticed that the peripheral injection cholic acids and sodium ferrocyanide had no effect on the CNS, while the intraventricular injection of the same compounds exhibits the specific pharmacological effects [2]. Therefore he concluded that the blood vessels in proximity to the brain selectively block certain substances, and he introduced the term "blood-brain barrier" to describe this mechanism.

Continued research by one of Ehrlich's students, Edwin Goldmann, unveiled a more complex grasp on the physiology of the BBB, published in several steps, most importantly in 1913 [3]. Staining studies with trypan blue showed a coloration of the brain when injected intraventricularly, but with intravenous injection no staining was observed except for the choroid plexuses. This demonstrated a separation between the CNS and the rest of the body and also that the choroid plexuses were gateways towards the brain.

Further observations were obtained by Lina Stern, which noted that an anti-tetanus drug administered intravenously did not cure tetanus once it affected the brain, thus concluded there is mechanism that protects the CNS from infectious and toxical compounds in the blood [4]. She introduced the term "hematoencephalic barrier" to describe an interface that completely blocks any molecular transfer towards the brain, a hypothesis proven improper years later.

THE DEMONSTRATION OF THE BLOOD-BRAIN BARRIER

The introduction of electron microscopy made it possible to directly visualize the structural composition of the BBB. The first studies by Edward Dempsey and his team, in 1955, demonstrated that the BBB should consist of one or more structures that separate the blood in the

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capillaries from the neurons, recte the capillary wall, the astrocytic feet and the basal lamina [5].

Subsequent studies published by Van Harrefeld in 1965-1967 used an improved method of fixation by rapid freezing on a metal plate and substitution fixation at low temperature shortly after acquiring the biological sample from the living organism. This procedure permitted the identification of the extracellular space in the CNS, which by conventional methods was destroyed and impossible to objectify [6].

Additional progress was recorded by Thomas Reese and his colleagues, in 1967-1969 when they demonstrated the actual role of interendothelial tight junctions, which surpassed the astrocytic feet and basal lamina in terms of significance [7].

Following studies using freeze fracture, performed by Nagy and his team and published in 1984, showed that tight junctions form a complex network restricting the passage of molecules up to 10-15Å [8].

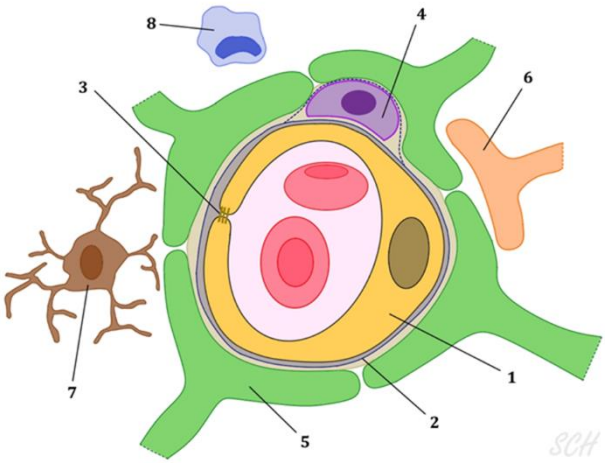

Fig. 1. The anatomical structure of the blood-brain barrier. 1. Endothelial cell. 2. Basal lamina. 3. Tight junction. 4. Pericyte. 5. Astrocyte foot. 6. Regulatory neuron. 7. Microglia. 8. Perivascular macrophage.

THE SPECIFIC ROLE OF INDIVIDUAL ANATOMICAL CONSTITUENTS OF THE BLOOD-BRAIN BARRIER

Endothelial cells

Studies have demonstrated that the most important component of the BBB is represented by the endothelial cells, which line the blood vessels and have morphological particularities that differentiate them from those that form somatic capillaries [9].

The endothelial cells in the brain capillaries have no fenestrations, hold more mitochondria compared to other endothelia in the body, and possess a continuous basal lamina to which they are attached through focal adhesions consisting of transmembrane proteins [10].

The fundamental role of the endothelial cells of the BBB is to block the access of hydrophobic intravascular components to the brain, and in the meantime allowing the regulated passage of key metabolites to the extracellular fluid and astrocyte feet while maintaining local hydroelectrolytic homeostasis [11].

Another important function of endothelial cells is to secrete the endothelial growth factor, brain-derived neurotrophic factor, insulin-like growth factor 1, substance P and other regulating factors [12-14].

Basal lamina

A crucial part of the BBB that surrounds and binds endothelial cells, the basal lamina is composed by multiple types of molecules such as collagen, elastin, proteoglycans, laminin, nidogen, osteonectin, perlecan and fibronectin [15].

The basal lamina plays a supportive role due to its composition rich in cell adhesion molecules that bind to the endothelial cells and surrounding structures such as the pericytes, astrocyte feet, and also contribute to the integrity of the tight junctions [16].

Tight junctions

One of the most important factors that reduce the permeability of various polar solutes and ions in their passage between the endothelial cells from the blood towards the brain [17].

Ultrastructurally, the tight junctions are composed of proteins and junction adhesion molecules developed in between two endothelial cells, or for the distal capillary, between the two extremities of the single endothelial cell composing the vessel wall [18]. The proteins that block the access of molecules through the tight junction are claudins and occludins, and not only their presence but their three dimensional organization are key factors to producing an impassable barrier [19].

Several other cytoplasmic proteins of the endothelial cells are indirectly involved in the composition of the tight junctions, which interact with the aforementioned membrane proteins, signaling them and regulating their expression [20].

The tight junctions are also the cause for the high electrical resistance of the blood-brain barrier causing a restriction of ion free motion [21].

Pericytes

Enveloping the capillaries of the BBB, the pericytes play a key role by regulating the vessel caliber, dynamic of contraction and structural stability, as well as secreting several angiogenic and differentiation factors [22].

The pericytes interact with the basement membrane, attaching themselves and dynamically interacting especially through the secretion by the pericytes of the

tissue inhibitor of metalloproteinase 3 and other protease inhibitors and enzymes involved in vascular modeling [23].

Endothelial cells secrete pericyte proliferation factors that favor their migration and development, and after their maturation they bind to the endothelial cells by means of transforming growth factor-β [24].

Several experiments demonstrated the increased resistance to apoptosis demonstrated by endothelial cells bound to pericytes, when compared to isolated endothelial cells, thus underlining yet another role of pericytes in the robustness of the BBB [25].

Astrocytes

The structural role provided by astrocytes resides mainly in nutritional support and the clearance of ions and metabolites. The astrocyte feet cover a significant circumference of the capillary surface and interact with adjoining neuronal bodies, endothelial cells and basal lamina, regulating their functions.

Animal experimental studies showed that astrocytes tend to develop around the endothelial cells and tighten the capillaries when transplanted into areas with leaky vessels, thus demonstrating an important role towards the induction and maintenance of the BBB [26].

In vivo studies evidenced the critical role of the astrocytes in the proper functioning of the BBB, studying the effect of selective destruction of astrocytes with hypoxic stress, which leads to the loss of endothelial tight junction protein expression, destroying the BBB. Restoration of the astrocytes in the affected area reestablished the normal parameters of the BBB [27].

Various signaling factors secreted by the astrocytes were identified, such as calcium, TGF-β, IL-6, and Glial cell-derived neurotropic growth factor (GDNF), some of them appearing to play a role in the modulation of the BBB phenotype, without actually being a factor in its structural resistance [28].

Regulatory neurons

Neurons do not play a direct role in the structure of the BBB, but act as modulators by releasing some enzymes regulating the endothelial cells, as well as conventional neurotransmitters such as norepinephrine, acetylcholine, GABA, serotonin and others [29].

Recent studies observed the disruption of the BBB through an increase of vascular endothelial growth factor release from the astrocytes activated by ischemic neurons, findings which were accompanied also by a decrease of occludin and claudin proteins that further diminished BBB integrity [30].

Neurons seem to have a signaling feedback with the endothelial cells, including secretion of proteins that promote synaptogenesis and the overall regulation of the cerebral blood flow, in response to fine changes in the neuronal activity [31].

Microglia

Microglia represent the effectors of the immunity system in the central nervous system, and can be subjected to various transformations in their activation process. The neuro-inflammation that appears is a common pathogenic pathway for numerous neurodegenerative diseases [32].

Several cytotoxic mediators are released by microglia during inflammation, including tumor necrosis factor α that easily destroys the BBB [33]. On the other hand, the close proximity of the microglia to the endothelial cells indicate their contribution to the properties of the BBB, yet the exact role they are playing is yet to be demonstrated.

Perivascular macrophages

Showing similar structure and immune phenotype as their blood-circulating counterparts, perivascular macrophages are a particular population of cells residing near the astrocytic feet that compose the BBB [34].

Perivascular macrophages can extend some branching processes that can help engulfing the vessels thus adding to the physical impermeability of the BBB, and some can even be found beneath the basement membrane, between the vessel and the astrocyte feet [35].

In certain infectious or inflammatory conditions, perivascular macrophages can accumulate in the interstitial spaces and either clear along with the acute process, or remain for a long and unspecific period of time [36].

There are several immunological roles of the perivascular macrophages that have been identified and they have also been implicated in the response to apoptosis, as well as in the phagocytosis and pinocytosis of various molecules [37].

THE REGULATION OF THE BLOOD-BRAIN BARRIER

More and more studies point out that there is a certain dynamics in the response of the BBB to various stimuli in physiologic and pathological states of the body. Regulating the strict permeability of the BBB is essential in the response to factors that can involve the central nervous system, and thus on one hand protecting the CNS from harmful molecules, and on the other allowing specific substances and cells to cross the barrier and facilitate the brain's response to aggression.

CONCLUSIONS

The blood-brain barrier is a very highly selective permeability barrier that uses different morphological and physiological mechanisms to separate the circulating blood from the central nervous system. The blood-brain barrier's constituents which are very heterogeneous in nature, ranging from nervous endings and nerve cells, vascular

cells and their connections, immune-response cells and others, help create a barrier that opposes its transition by mechanical, electrical, chemical and immune forces, all while being strictly regulated by the central nervous system. The understanding of the blood-brain barrier is critical in order to control its functions in pathological conditions, intentionally modifying the permeability for specific substances.

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ÎNȚELEGEREA BARIEREI HEMATO-ENCEFALICE

REZUMAT

Bariera hemato-encefalică este o structura cvasi-impenetrabilă ce separă sângele de sistemul nervos central. Existența ei a fost dedusă de oameni de știință încă din secolul 19, însă, abia la mijlocul secolului al 20-lea, progresul tehnologic a permis vizualizarea directă a constituenților acesteia. Studiul barierei hemato-encefalice continuă și în prezent, din cauză că nu toate rolurile componenților acesteia au fost înțelese pe deplin, noi informații fiind descoperite prin explorări în situații atât fiziologice cât și patologice, in-vivo și in-vitro. Celulele endoteliale și joncțiunile lor strânse, lamina bazală, terminațiile astrocitare, pericitele și celelalte porțiuni structurale ale barierei hemato-encefalice contribuie la gradul acesteia complex de impermeabilitate prin factori mecanici, electrici, chimici și imuni.

Cuvinte cheie: bariera hemato-encefalică, ultrastructură, neurofiziologie

BIOCHEMICAL STUDIES OF AQUEOUS EXTRACT OF GARLIC ON THE MYOCARDIUM OF LEFT VENTRICLE OF HIGH SALT FED ADULT WISTAR RATS

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ABSTRACT

Background: This study was designed to demonstrate the restorative effect of garlic extract on the biochemical activities of the myocardium of left ventricle of high salt fed adult Wistar rats.

Methods: Twenty-five healthy female Wistar rats weighing 130-180 g were randomly assigned into 5 groups of 5 rats each (Groups A, B, C, D and E). Rats in group A were fed with standard laboratory pellets, while groups B, C, D and E were fed on the high-salt diet for five weeks. Thereafter, daily administration of 50 mg kg^{-1}, 100 mg kg^{-1} and 150 mg kg^{-1} of the garlic extract were giving orally to groups C, D and E respectively for 3 weeks while rats in groups A and B were administered distilled water. The rats were sacrificed under ketamine anesthesia (30mg/kg i.m). Blood samples were collected for the measurement of serum electrolytes and part of the heart ventricle was homogenized for the determination of lactate dehydrogenase activity. One-way ANOVA was used to analyze data, followed by Student Newman-keuls (SNK) test for multiple comparison.

Result: Result showed that the LDH activity, plasma level of sodium and potassium of group B rats were significantly higher ($P < 0.05$) when compared with the control group.

Conclusion: The study concluded that aqueous garlic administered altered the above-mentioned biochemical parameters toward normal value; this indicated that it contains the restorative effect on myocardium disorder.

Keywords: biochemical, high salt fed, myocardium, garlic

INTRODUCTION

Salt intake observed more than a half century ago a dose-dependent decrease in survival of rats fed a standard diet containing increasing amounts of salt [1]. Research showed that, it increased mortality by accelerating arteriosclerosis, renal parenchymal damage and cardiovascular disorders. These outcomes were associated with changes in blood pressure. This dramatic illustration of the effect of excess salt intake on mammalian cardiovascular and renal physiology has not been stressed, but recent developments have revitalized these original findings, with emphasis on the role of the renin-angiotensin aldosterone system (RAAS) in cardiovascular disease [2].

Garlic had been reported to have prophylactic, restorative and curative properties in various conditions such as microbial infection, thrombosis, hypertension, hyperglycemia, hyperlipidemia, cancer, and thrombosis [3,4]. The contents of the extract include sulfur active principles mainly in the form of cysteine derivatives such as s-alkyl cysteine and sulfaxides, which decompose into a variety of thiosulfinates and polysulfides by the action of an enzyme allinase on extraction [5]. Evidence from numerous studies has pointed out that garlic can bring about plasma lipid normalization, enhance fibrinolytic activity and inhibit platelet aggregation and thromboxane formation [4]. Several reports have suggested that garlic has protective effect against stroke and atherosclerosis [6]. Fresh garlic extracts have been found to lower blood pressure in spontaneously hypertensive rats and in anesthetized dogs [7].

High prevalence of cardiovascular disease, side effect of available antihypertensive drugs, recent trend in any development from medicinal plant, this study investigated alternative therapy that are likely to be more effective and cheaper than the current therapies. The goal of this study was to show that garlic extract still possess the restorative effect on the biochemical activities on the myocardium of left ventricle of high salt fed (HSF) adult Wistar rats.

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MATERIALS AND METHOD

Animal Care and Management

Twenty-five healthy female Wistar rats weighing 130-180 g obtained from the Animal Holding of College of Health Sciences, Obafemi Awolowo University Ile-Ife were used for this research. The rats were randomly assigned into 5 groups of 5 rats each (Groups A, B, C, D and E). They were maintained on standard laboratory rat pellets before the commencement of the experiment and water was provided *ad libitum*.

Plant Material and Preparation of Extract

Cloves of garlic bulb were procured from Sabo market in Ile-Ife and identified by a taxonomist in the Department of Botany, Obafemi Awolowo University, Ile-Ife. They were weighed and then blended with water. After which they were filtered into a conical flask using a filter paper and a funnel to produce a clear juice. Then, the filtrate was freeze-dried using lyophilizer and stored in a desiccator. An aliquot portion of the crude extract residue was dissolved in distilled water which was been used on each day of the experiment [8].

Preparation of High Salt Diet (HSD)

HSD containing 8% sodium chloride was prepared specially by replacing 0.3% sodium chloride-containing standard diet with 8% sodium chloride [9,10].

Animal Treatment

Group A was the control, group B was negative control, while groups C, D and E were the test groups. Rats in group A were fed with standard laboratory pellets, while groups B, C, D and E were fed on the high-salt diet for five weeks. Thereafter, daily administration of 50 mg kg^{-1}, 100 mg kg^{-1} and 150 mg kg^{-1} of the garlic extract were giving orally to groups C, D and E respectively for 3 weeks, while rats in group B was left untreated for the same period. The extract solution was administered orally, using oral cannula and duration of the experiment was 8 weeks.

Sacrifice of Animals

At the end of the experiment, the rats were sacrificed under ketamine anesthesia (30mg/kg i.m). Blood samples were collected for the measurement of serum electrolytes and part of the heart ventricle was homogenized for the determination of lactate dehydrogenase (LDH) activity.

Homogenization of Myocardium of Left Ventricle of the Heart

The tissues were excised, weighed and were chopped into small pieces with a scalpel holder and blade. Each of the tissues was washed three times in ice-cold 0.9% KCl. All subsequent manipulations were done at 4°C. The tissues were then homogenized in Teflon glass homogenizer within ten fold volumes excess of 5 mm TRIS homogenization buffer (PH 7.7 at 2°C). The homogenization buffer contained 3.338 mm TRIS HCL, 1.669 mm TRIS base, 250 mm sucrose and 2 mm EGTA, The homogenates were centrifuged at 5000 rpm for 5 minutes to obtain clear supernatant. The supernatant was then assayed for the levels of activity of lactate dehydrogenase enzymes using Randox kit for LDH (Randox Laboratory, Northern Ireland).

Measurement of Serum Electrolytes

Blood sample from each rats was collected separately into clean capped plain tubes and allowed to stand for 30 minutes for clotting to occur. These were then centrifuged at 2500 revolution per minutes for 15 minutes. The serum was extracted into clean test tube for the analysis of sodium and potassium. This was measured using Flame photometry method at wavelength of 590 nm for sodium and 770 nm for potassium.

Photomicrography

Stained sections were viewed under a LEICA research microscope (LEICA DM750, Switzerland) with digital camera attached (LEICA ICC50) and digital photomicrographs were taken at various magnifications.

RESULTS

One way ANOVA revealed that there was significant difference ($F_{4, 20}$ = 4.217; p = 0.0174) in activity of LDH across all experimental groups. Post-hoc analysis showed that LDH activity of HSF group was significantly higher (p = 0.0174) than the control group. The LDH activity which was induced by HSD was significantly reversed by garlic extract. The 100 mg/kg of garlic extract produced the highest level of reversal, which was not significantly different when compared with control (Figure 1). Post-hoc analysis showed that serum sodium ion concentration of group B was significantly higher than the control group (p<0.05). The increased serum sodium ion concentration which was induced by the HSD was reversed by garlic extract in groups C, D and E (Table I). There was significant difference in serum potassium ion concentration across all experimental groups ($F_{4, 20}$ = 28.54; p<0.05). Serum potassium ion concentration of group B was significantly lower than the control group (p<0.05). The serum potassium ion concentration which was lowered by the HSD was significantly and dose-dependently reversed by extract in groups C, D and E. However, the highest dose of garlic used in this study (150 mg/kg) did not produce a complete reversal of HSD-induced hypokatrimia to such a level that was significantly higher than the vehicle group (p<0.05) (Table II).

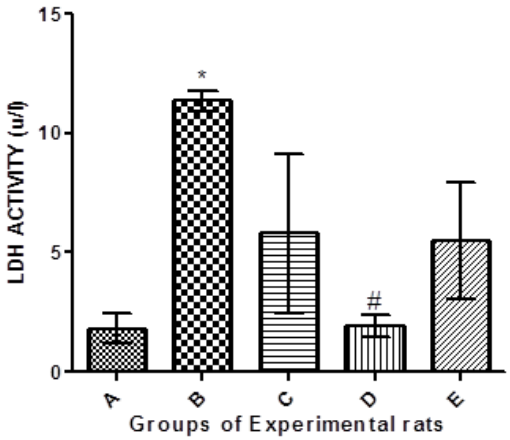

Fig. 1. Effect of Aqueous Extract Garlic Activity on LDH of Myocardium of Left Ventricle of Rats Fed with HSD; n = 5, values are expressed as LDH Activity (u/l) ± SEM, *relative to control at $p<0.05$; # relative to group B (HSF) at $p<0.05$.

Table I. Effect of Aqueous Garlic Extract on Serum Sodium Ion of Rats Fed with HSD; n = 5, values are expressed as serum sodium (mmol/L) ± SEM, *relative to control; # relative to group B (HSF) at $p<0.05$.

Groups	Serum sodium ion (mmol/L) ±SEM
A	137.30±0.42
B	144.90±0.06*
C	141.80±0.19*#
D	141.10±0.27*#
E	139.10±0.02#

Table II. Effect of aqueous garlic extract on serum potassium ion of rats fed with HSD; n = 5, values are expressed as serum potassium (mmol/L) ± SEM, * relative to control; # relative to group B (HSF); a relative to group C (HSD + 50 mg kg^{-1} of garlic extract); b relative to group D (HSD + 100 mg kg^{-1} of garlic extract); $p<0.05$.

Groups	Serum potassium ion (mmol/L) ±SEM
A	4.49±0.18
B	3.95±0.02*
C	4.03±0.02*
D	4.67±0.04#a
E	4.99±0.02*#ab

DISCUSSION

Biochemical studies of aqueous extract of garlic on the myocardium of left ventricle of HSF adult wistar rats were carried out in this study. It was observed that the lactate dehydrogenase (LDH) was significantly higher in the HSF group than the control group ($p<0.05$). Similarly, the concentration of plasma sodium and potassium ion was significantly higher in HSF group than the control group ($p<0.05$). This suggests that HSD resulted in hyperkaliemia, hypernatrimia and cardiovascular disorder in myocardium of left ventricle of the heart. High salt intake promotes the elevation of blood pressure, cardiac hypertrophy, the impairment of left ventricular relaxation, endothelial dysfunction and kidney injury [8], while some studies have linked the HSD–induced cardiovascular disorder to salt-induced hypertension [9]. Increasing evidence from multiple clinical studies showed that excess salt intake was related to cardiovascular organ damage, independent of blood pressure [10]. The pathophysiological mechanisms responsible for high salt diet–induced remains debatable.

It is well known that lactate dehydrogenase (LDH) is one of the diagnostic marker enzymes of myocardial damage. Presence of this biomarker in heart tissue homogenate (HTH) is indicative of myocardial integrity and their release in serum signifies myocardial injury. The results of this study showed that LDH activity increased in the HSD. However, garlic extract has restorative effects on high salt induced cardiac damage as revealed by reduction in the concentration of LDH of treated groups especially those with 100 mg kg^{-1} and 150 mg kg^{-1} of garlic extract. It was reported that garlic consumption had significant restorative effect on both animals and human [11]. It was also reported that aqueous garlic extract was beneficial in ameliorating LDH activity in Lead-induced oxidative damage in the rat heart [12].

The active ingredient in garlic extract is known as Allicin (diallyl thiosulfinate) which is a mainly organosulfur [13] had been shown to reverse the effect of high salt in this research. Garlic had been reported to exhibit potent angiotensin converting enzyme (ACE) inhibitory activity [14]. The reduction in ACE activity results to reduction in plasma level of angiotensin-II. Angiotensin II is also known to cause cardiovascular remodeling [15]. Garlic extract-induced inhibition of ACE probably leads to reduction in angiotensin-II, leading to decrease adrenal production of aldosterone [16]. The Garlic extract-induced reduction of plasma level of angiotensin-II followed by natri- and diuresis may be responsible for the reversal of the high salt-induced cardiovascular disorder.

CONCLUSION

The result of this study indicated that high salt diet causes significant histomorphological changes on myocardium of left ventricle of rats as evidenced by significant increase of LDH, serum sodium and potassium. Garlic extract has restorative and ameliorative properties on these high salt diet-induced changes in cardiovascular system.

CONFLICT OF INTEREST

There was no conflict of interest among the authors and every necessary detail was agreed upon during the preparation of the work.

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AGE- AND DOSE-DEPENDENT HAEMATOLOGICAL CHANGES IN LACTATIONAL LEAD-INTOXICATED RATS

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ABSTRACT
Objective: High to low lead (Pb) concentrations in breast milk has been found to perturb some biological events in the postnatal life. While postnatal Pb exposure has been reported to impair some haematological parameters in mammals, the age-dependent haematological signature of lactational Pb poisoning is not clear. This study investigated the effects of Pb exposure during lactating period on the haematological profile of rats at certain post-lactational ages using varying doses of Pb.
Methods: Lactating mothers and their pups were randomly divided into 4 groups comprising 24 pups each. The treatment groups received 10 mg/dL, 30 mg/dL, and 70 mg/dL of lead acetate in their drinking water from postnatal day one (P1) to P21 of the lactating period. The control rats received distilled water. At P22, P60, P90 and P120, the pups from each group were euthanized and blood was collected and analyzed for blood Pb levels, packed cell volume (PCV) and total white blood cell (WBC) count.
Results: Lactational lead poisoning resulted in higher blood lead levels among the exposed pups ($p < 0.01$ compared with controls); the PCV did not show statistically significant differences from the controls until P90 and P120. Moreover, WBC count was significantly higher ($P>0.05$) from the control as an initial response to Pb intoxication except in the group treated with the highest Pb dose, where WBC count was significantly reduced.
Conclusion: We concluded that lactational Pb intoxication is a means of Pb poisoning in rat pups, and that high concentration of Pb in maternal breast milk is associated with reductions in WBC counts in the pups. High lactational Pb is also associated with post-lactational impairment of the immune system and anemic responses in rat pups.
Keywords: lead poisoning, lactational, postnatal, haematological, RBC, WBC.

INTRODUCTION

Lead poisoning (also known as plumbism, colicapictorum, saturnism, Devon colic, or painter's colic) is a medical condition in humans and other vertebrates caused by increased levels of the heavy metal lead (Pb) in the body, that is, an intoxication resulting from absorption of hazardous levels of lead into body tissues [1,2]. Pb is well-known as a major global environmental hazard. The Centre for Disease Control (CDC) [4,5] currently considers lead poisoning the leading environmental health threat to children in the US [3]. No safe threshold for lead exposure has been discovered; that is, there is no known sufficiently small amount of lead that will not cause harm to the body. The Centers for Disease Control [4,5] (US) has set the standard of elevated blood lead level for adults to be 10 µg/dl of the whole blood. For children the number is set much lower at 5 µg/dl of blood as of 2012, down from a previous 10 µg/dl [4,5].

The alterations in hematological changes serve as the earliest indicator of toxic effects on tissue [6]. Anemia may result when the cell membranes of RBCs become more fragile as a result of damage to their membrane [7]. According to Harkness and Wagner [8], mean hemoglobin levels for rodents vary from 10 to 17 g/dL. High levels of lead exposure during gestation and lactation can severely damage heme synthesis [9]. Hallen *et al.,* [10] established that lactational lead exposure results in far higher (6 times) intoxication than placental intoxication. Maternal Pb can also be transferred to infants during breastfeeding. The placenta and breast ensure that the developing fetus and young child are exposed early [11].

While studies on the impact of lactational Pb intoxication on haematological indices remain very scanty, most previous studies were limited to only one dose, and studies limited to a particular period post weaning. This study was therefore designed to investigate the effects of lactational lead intoxication on pups' packed cells volume (PCV) and total white blood cells count at different ages and doses following weaning.

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MATERIALS AND METHODS

Chemicals

Lead II Acetate trihydrate ($Pb(CH_3COO_2).3H_2O$) was a product of Guangzhou Jinhuada Chemical Reagent Co., Ltd, China. Other chemicals and reagents were of analytical grade and were procured locally.

Animals and lead acetate treatment

Wistar rats bred in the animal holdings of the Department of Anatomy, Bowen University Iwo, Osun state, Nigeria were used for this study. Sexually mature rats (120-150 days old) were mated under standard laboratory conditions at a constant light/dark cycle. The pregnant rats were isolated into separate cages and fed with standard rat chow from Ladoke Feeds, Ibadan and water was supplied *ad libitum.* The day each dam was littered was marked day 0.5.

Throughout the lactating period (3 weeks postnatal), 3 groups of nursing dams were exposed to varying doses of lead acetate in their drinking water [lower dose: 10 mg/dL [12]; moderate dose: 30 mg/dL [13]; and higher dose: 70 mg/dL [14]. We expected that the pups were indirectly exposed to Pb via the dams' breastmilk [11]. Control dams were administered distilled water, and so their pups had no lactational Pb exposure.

Collection of biological samples

At postnatal day 22 (P22), i.e. one-day post-weaning, P60, P90 and P120, pups (n = 6) from each group were euthanized using 40 mg/Kg body weight sodium pentobarbital (IP). The total body length and whole body weight for each animal were determined. The thorax was exposed to gain access to the heart; cardiac puncture was performed to collect blood into heparinized specimen bottles.

White blood cell count

This was done with Hawskley haemocytometer (Marl Borough business park, lancing, UK).). The white bead pipette is filled with blood up 0.5 marking. It is then diluted with Turk's solution (1:20) to the 11th gradation on the pipette. The diluted fluid was then released unto the counting chamber and covered with cover slip. The counting chamber was then placed under the microscope and examined with scanning (X4), low power (X10), and high power (X40) objective lens through which the counting was done. Results were expressed as counts per mm^3.

Packed cell volume (PCV)

In the determination of PCV, the heparinized capillary tube was filled with blood through one end with the other end secured with plasticine. The capillary tubes were then placed in the micro hematocrit centrifuge, well counter balanced and spun for 15 minutes at 3000 revolutions per minute (rpm). Results were expressed in percentages.

Quantification of blood lead

This procedure was carried out to establish the concentration of lead in the blood of the pups at the point of sacrifice. For each of the rats, a sample solution of whole blood and 2M concentrated H_2SO_4 was prepared by diluting into a specimen bottle, 1ml of whole blood with 3ml of 2M concentrated H_2SO_4 (ratio 1:3). The acid was used to digest the red blood cells to release lead within the blood cells. The solutions were centrifuged for 15 minutes at 4000 rpm. Each supernatant was decanted into a new heparinized specimen bottle and the centrifugation was repeated again to ensure that the supernatant was clear. The clear supernatants were decanted into specimen bottles and each sample was analyzed using the atomic absorption spectrometer (AAS). The AAS was set at the standard wavelength for lead analysis; 283.3nm. Five standard lead analysis solutions (0, 2.5, 5, 10 & 20 ppm) were run through the AAS followed by each sample. The lead concentration in the serum was recorded by the AAS.

RESULTS

There was statistically significant difference between the control (0.237 ± 0.036; 0.541 ± 0.123; 0.633 ± 0.314 and 1.547 ± 0.567, Mean ± SEM) and the treated groups 10mg/dl (7.330 ±1.965; 5.928±1.013; 5.092±0.962 and 4.568±0.009 Mean ± SEM); 30mg/dl (20.722 ±1.631; 15.416±1.844; 7.356±1.356 and 5.120±0.267, Mean ± SEM) and 70mg/dl (26.686 ±1.548; 20.656±1.712; 15.782±0.478 and 12.187±0.115, Mean ± SEM) at the different stages of investigation (Postnatal days 22,60,90 and 120), $P<0.01$. Although almost negligible when compared with the treated animals, the control animals still showed some levels of Pb in their blood; this shows the persistence of Pb presence in the environment. * represents statistical significance at $P>0.01$ compared with the control (Figures 1 and 2).

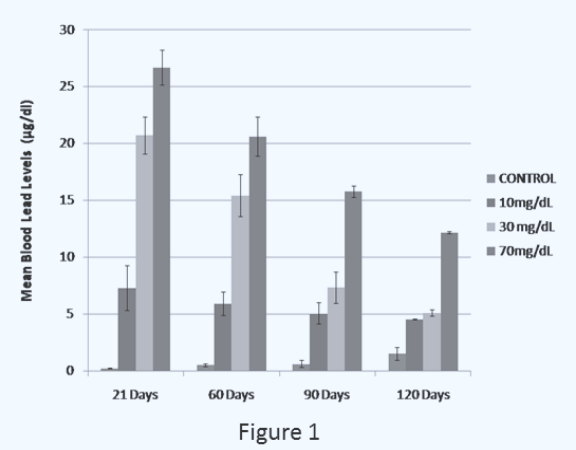

Fig. 1. Mean blood lead levels (µg/dl)

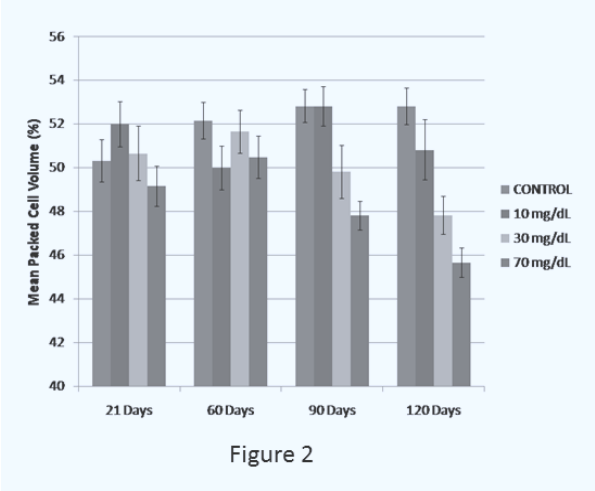

Fig. 2. Mean packed cell volume (%)

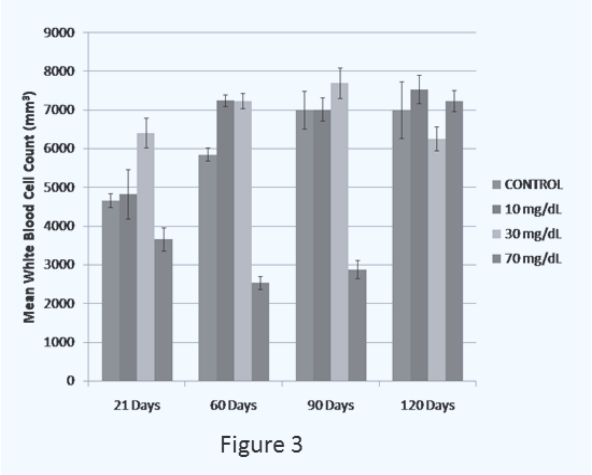

Fig.3. Mean white blood cell count (mm^3)

The percentage reduction in BLL within each group at P60, P90 and P120 from P21are:10mg/dl (68%; 42% and 20%); 30mg/dl (57%; 25% and 10%) and 70mg/dl (64%; 37% and 16%) respectively. This shows closely related but variant rates of body Pb elimination across the groups (Figure 3).

Effects of Lactational Lead Exposure on Packed Cell Volume in Rats

The PCV in the control and the treated groups initially showed some level of constancy that is not significantly different at postnatal days 22 and 60, until postnatal day 90 where 30mg/dl and 70mg/dl showed some statistically significant low levels of PCV, and postnatal day 120 when all treated groups expressed some statistically significant low levels of PCV compared with the control.

No statistically significant difference was recorded among the treated groups and the control at P22 and P60 ($p>0.05$). Compared with the control (52.830 ± 0.749), 30mg/dl (49.830 ± 1.222) and 70mg/dl (47.830 ± 0.654) groups were significantly lower at P90, while all treated groups showed statistically significant low PCV at P120 compared with the control (52.830 ±0.833); 10mg/dl (50.830 ± 1.376); 30mg/dl (47.830 ± 0.872); 70mg/dl (45.670 ±0.667). * represents statistical significance at $p>0.05$ compared with the control (Figures 4 and 5).

While the control group showed initial increase from P21 to P60 and subsequent stability, 10 mg/dl group was unstable while 30 mg/dl and 70 mg/dl groups showed initial increases from P21 to P60 with subsequent statistically significant decreases in PCV as presented above. $p>0.05$

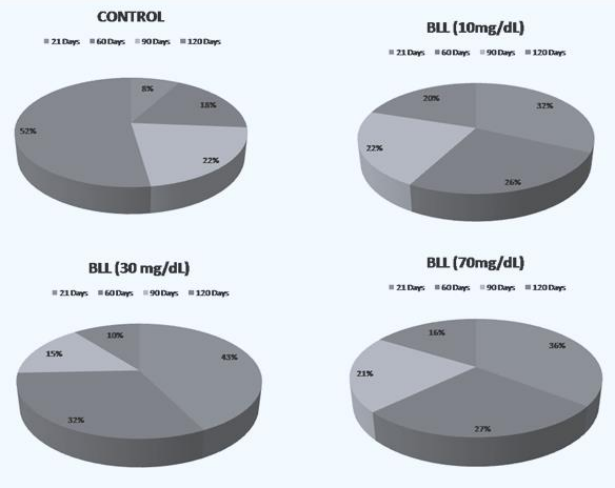

Fig. 4. Control vs. BLL

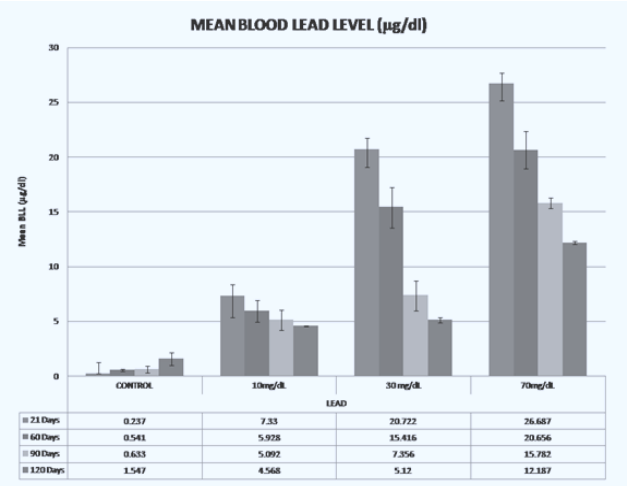

Fig. 5. Mean blood lead level (µg/dl)

Effects of Lactational Pb Exposure on Total White Blood Cell Counts in Rats

The initial reaction of white blood cells to lead was observed as significant level of leukocytosis in 30mg/dl group and slightly seen in 10mg/dl group (not statistically significant), by postnatal day 60, the two groups had shown statistically significant increase in total white blood count. By postnatal day 90, the 10mg/dl group had fully recovered

while the 30mg/dl group was still slightly higher than the control group but none was statistically significant. Up till postnatal day 90, the 70mg/dl group was consistently leukopenic at a statistically significant level when compared with the control. By postnatal day 120, all the treated groups exhibited no significant difference from the control.

Only the 30mg/dl group (6408.33 ± 386.095) showed significantly higher total WBC count at postnatal day 21 compared with the control (4666.67 ± 180.123). At P60, the 10mg/dl group (7247.17 ± 155.996) and 30mg/dl group (7238.33 ± 201.501) showed statistically significant higher levels of total WBC count compared with the control (5858.33 ± 166.542), whereas these groups showed no statistically significant difference from the control at P90 and P120. At P21, P60 and P90, the 70 mg/dl group (3666.67 ± 299.692; 2541.67 ± 159.904 and 2883.00 ± 231.541, respectively) showed statistically significant lower total WBC compared with the control, but not significantly different from control at P120. * represents statistically significant difference at p>0.05 compared with the control (Figures 6 and 7).

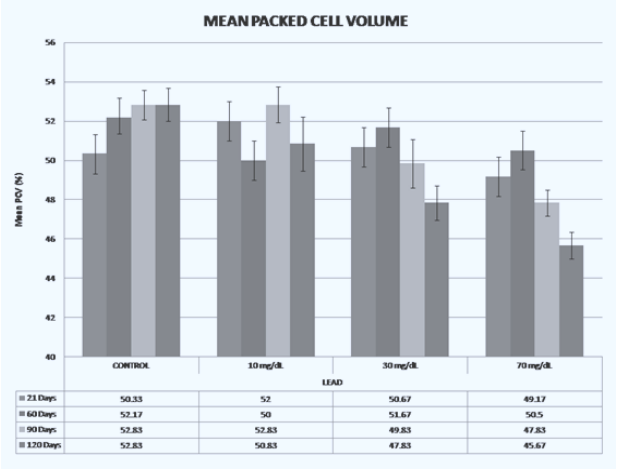

Fig. 6. Mean packed cell volume

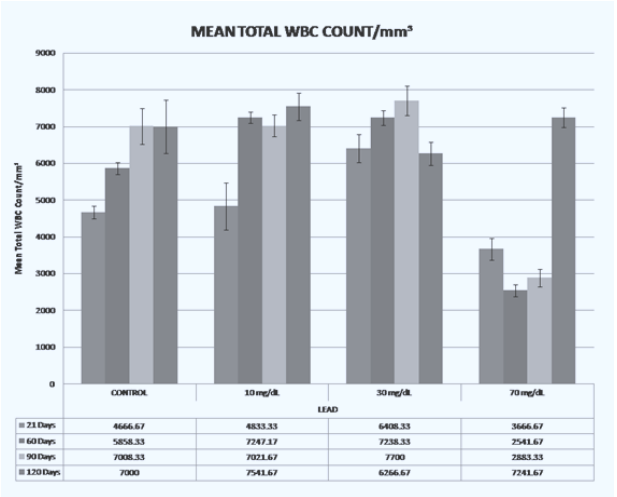

Fig. 7. Mean total WBC / mm^3

DISCUSSION

This study evaluated age-dependent haematological characteristics in lactational Pb-exposed rats at certain postnatal time points. We have shown that while PCV remained largely undisturbed at 21 and 60 postnatal days in rats exposed to lead via the breast milk, total white blood cell counts were significantly reduced in the early postnatal period in these pups, especially at the highest Pb dose of 70 mg/dl. Lower doses of Pb showed significantly higher total WBC count, a probable initial response to Pb induced inflammation.

Hallen *et al.,* [10] reported that lactational lead exposure results in far higher (6 times) intoxication than placental intoxication. Hua-Wang [15] also showed that there is only a difference of 3% between blood lead levels and breast milk lead levels in rats [16,17]. It thus implies that lead concentrations in breast milk are almost as high as lead concentrations in blood. Hence, in our study, we anticipated that the high blood lead levels recorded by means of atomic absorption spectrometer was suggestive of proportionally similar lead levels in the exposed dams' breast milk, and by extension, in the pups fed from sucked Pb-contaminated milk.

Several hematological perturbations have been recorded sequel to lead intoxication. Kales *et al* [18] showed that lead poisoning was associated with basophilic stippling, low hemoglobin and higher protoporphyrin. Findings from studies by Yu [7] also showed that the initial impact of lead poisoning is to alter the cell membrane of red blood cells, and it was subsequently demonstrated by Patrick [19] that 99 % of the circulating lead in blood is found in red blood cells and only 1% in the plasma. Therefore, the absence of significant differences in PCV between the control and lead-treated groups in the present study further suggest that the initial impact of lead poisoning on RBC is more of qualitative (morphologic) perturbations rather than quantitative impact, as initially reported by Sachdev and Veena [9].

On the other part, statistically significant differences in the PCV were observed at the longer duration post-exposure (90 and 120 days). This might be an indication of hematopoietic perturbation. It has been established that the half-life of lead in the bones may be as long as 20-30 years in man, but only weeks to months in soft tissues and about 30 days in the blood [20]. Lead also inhibits ferrochelatase (haemsynthetase) which catalyses the introduction of ferrous iron into the porphyrin ring to form heme, a process in haematopoiesis [21].

Lead poisoning has been implicated in anemia, reticulocytosis, and basophilic stippling since the beginning of the century. Lead binds copiously to the red cells [22] with about 50 times as much found within the bone marrow [23]. These abnormalities are more pronounced in the erythroid series, especially ineffective erythroid hyperplasia [18].

When lead enters into a biological system either through skin absorption, inhalation and/or ingestion, the first place of call is the blood which would circulate it round the body and it is first deposited in soft tissues and then hard tissues e.g. bones, teeth [6,24]. The bones eventually harbor the highest level of lead. This means that high level of lead in the bones would potentially disrupt the normal process of hematopoiesis in the bone marrow, and this may proceed for a long time due to slow deposition of lead in bones [19,25,14].

On the other hand, Peris and Salibian [26] reported significant increase in reticulocytes as an early response to Pb intoxication. Noori Mughai *et al.,* [27] associated this increase in total leukocyte count mainly to increase in nuetrophil and monocyte counts, and an insignificant increase in lymphocytes count. This early response to Pb intoxication was explained by Yagminas *et al.,* [28] as changes due to Pb induced inflammation. These findings are only consistent with our findings in the 10 mg/dl and 30 mg/dl groups.

Noori Mughai *et al.* [27] reported initial thrombocytopenia after intoxication followed by thrombocytosis. Sudakova *et al.* [29] reported leucopenia (low WBC count) as an initial response in acute plumbism which was consistent with our findings in the 70 mg/dl treated group, they reported a disappearance of this low WBC count after acute plumbism was resolved which also was consistent with our findings in this group.

CONCLUSION

This study has been able to demonstrate that Pb exposure during lactation period is a means of Pb poisoning in rat pups, and that high concentration of Pb in maternal breast milk is associated with reductions in WBC counts in the pups at each of the days studied. These findings suggest that high lactational Pb is associated with post-lactational impairment of the immune system and anemic responses in rat pups.

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INTEGRATING PHYSIOLOGY IN THE "URINARY SYSTEM" MODULE

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ABSTRACT
Victor Babes University of Medicine and Pharmacy Timisoara ran a project which included a series of extracurricular modular courses to evaluate the impact of integrated medical curricula. "The Urinary System" was part of this effort. The modular course ensured vertical and horizontal integration in order to provide students with thorough understanding of kidney structure and functions, as well as selected features of various kidney pathologies and was offered as two summer school sessions (in 2013 and 2015 years). The course consisting of lectures and practical applications was scheduled for a week, also including workshops and small group discussions in a problem-based learning setting. A total number of 66 students were selected based on letters of intent and interviews. At the end of the summer school, students' knowledge was assessed by a multiple choice question (MCQ) test, while the modular course itself was evaluated by students through a questionnaire. MCQ results were above average, which was expected since this was a free chosen activity by students who were among the first 10% of their respective year of study. At the end of the course, the students were able to discriminate between normal and pathological results and to correlate clinical presentation with results, and acquired a better understanding of the newest developments in the field, while the teachers gained experience in working together. Overall, the summer school experience was highly appreciated, as seen from the analysis of answers to the questionnaires for students and teachers. Conclusions: (1) Small group teaching enhances the quality of teaching and learning processes. (2) Close interaction with students enables appropriate feedback for improvement of teaching process. (3) An integrated approach to teaching and learning allows students to better integrate pre-clinical knowledge into clinical context.
Key words: medical education, integrated modular course, small group discussions

INTRODUCTION

Medical education programmes worldwide differ remarkably, with a wide range of approaches on such aspects as quality standards, curricula or methods of delivering education. World Health Organisation acknowledged that the great differences in the contents of medical curricula, leading to a high variation in the competences of medical graduates, should be reduced by setting "Global Minimum Essential Requirements" for medical professionals across the world [1]. There has been much debate about the quality of medical education during the last decades, centred on the idea of connecting education with society needs by tailoring appropriate medical curricula [2] and promoting competence-based education [3].

An integrated medical curriculum would serve the ultimate goal of preparing medical graduates able to cope with current healthcare demands. Integration of medical curriculum, either horizontal or vertical, or both has been already implemented or is in progress in many medical schools around the world, although large differences between countries exist, with the highest percentages of schools delivering integrated curricula found in the developed countries [4]. The aim of integration is to bring together basic and clinical sciences across the entire curriculum in order to enable the medical student to concurrently develop theoretical knowledge and practical skills [5].

Horizontal integration, i.e. between disciplines in the same phase of the traditional curriculum (either pre-clinical or clinical) and vertical integration of disciplines in different phases (pre-clinical and clinical) [6] are both required to achieve a fully integrated medical curriculum. Designing and implementing a modular curriculum is a way to accomplish integration at all levels, as several disciplines are grouped under a single module. Nevertheless, it requires careful planning of modules in the curriculum in order to reach the desired educational outcomes [7].

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DESIGN

Such an approach was attempted by the Victor Babes University of Medicine and Pharmacy Timisoara (UMFVBT) within the eMediqual (European Quality and Professional Competence in Medical University Education and Management', Sectorial Operational Programme Human Resources Development 2007 – 2013, ref. POSDRU 86/1.2/S/63815) project (2011 – 2013) in which curriculum development was defined as a general objective. Several extracurricular modular courses were designed to evaluate how an integrated medical curriculum would improve the quality of both teaching and learning. One of these was "The Urinary System", a summer schools offered by UMFVBT to students enrolled in the 2nd to 5th year of study. It was first organised in 2013 as a task in the project and then again in 2015 due to its success among students.

The modular course was designed to ensure both vertical and horizontal integration in order to provide students with a thorough understanding of kidney structure and functions, as well as selected features of various kidney pathologies. Horizontal integration was achieved by an interdisciplinary approach, i.e. bringing together pre-clinical disciplines (Anatomy, Histology, Physiology, Pathophysiology and Microbiology) on the one hand, and clinical ones on the other hand (Nephrology and Urology), with Semiotics linking the two levels. Vertical integration between pre-clinical and clinical disciplines was further supported by adding paraclinical disciplines (Clinical Laboratory and Medical Imaging). Case scenarios provided within workshops and small group discussions served the aim of applying integrated knowledge acquired from pre-clinical, paraclinical and clinical disciplines. Even immunology basics were integrated with urology and nephrology in the context of kidney transplantation.

Six months before the first session of the actual course (July, 15-20, 2013), a team of 18 professors started to develop the summer school programme during a series of meetings in which they formulated the scope of integration, learning objectives and strategies, themes, teaching methods, clinical cases and assessment method. This team work facilitated integration between pre-clinical, paraclinical and clinical knowledge, ensuring adequate and relevant information was provided to students in order to help them acquire a comprehensive view on the normal and pathological kidney function, as well as to develop their problem-solving skills and clinical abilities.

Ten major themes, starting with the study of nephron and ending with the management of renal patient, were built around several case reports presented to students as a premise of teaching and ground of discussion. Seven hours of lectures and practical applications were scheduled each day, with lectures followed by workshops and small group discussions or practical works. The entire module was conceived so as to allow the use of both expository and interactive teaching methods, with problem-based learning at the core of the educational approach. At the very beginning of the modular course, the students were confronted with a case scenario (Box 1) that needed to be solved lately, after they would have acquired enough knowledge to attempt a diagnosis. This set the stage for the theoretical information delivered during the week in the form of lectures. Physiological and pathophysiological mechanisms described in the first part of the module were then used to build knowledge on various renal pathologies. The goals were to facilitate student understanding of the urinary system and normal vs. altered functions, and to provide theoretical background and practical tools that would allow them to recognise pathological changes involved in various kidney diseases.

Box 1.
Case scenario – Diabetes-associated chronic kidney disease

A 42-year-old male patient presents with a complaint of polyuria and xerostomia aggravated during the last 6 months. He is overweight (BMI 27 kg/m^2), his blood pressure is 170/100 mmHg, heart rate 80 bpm, sinus rhythm.

He has irregular eating habits with frequent consumption of fast-food meals and carbonated drinks, a sedentary lifestyle and he is a smoker.

One month before presentation his health status was evaluated at work: blood pressure 145/70 mmHg, heart rate 80 bpm, sinus rhythm, fasting glucose 105 mg/dl.

Current laboratory data: blood glucose 225 mg/dl, HbA1c 11.1%, cholesterol 241 mg/dl, HDL-C 37 mg/dl; triglycerides 369 mg/dl, LDL-C 130 mg/dl, TGO/AST 19 U/L, TGP/ALP 30 U/L, creatinine 1.40 mg/dl, urea 60 mg/dl, eGFR/MDRD 56 ml/min/1.73m^2; ACR 300 mg/g urinary creatinine; ionogram within normal limits.

Workshops and small group discussions

Workshops were focused on specific topics and new case scenarios were introduced to students which were afterwards solved during the small group discussions part. For example, the Physiology workshop "Functional Exploration of the Kidney" was designed around four themes: *Fluid and Electrolyte Balance, Acid-Base Balance, Urinalysis* and *Renal Excretion of Protein Catabolic By-Products*. Theoretical information, including normal values for each of the parameters were provided and multiple-choice questions were asked at key points to help students summarize and self-assess their learning progress (see Box 2 for an example). Students were encouraged to collectively find the correct answer by analysing and correlating case data as compared with normal parameters and had to explain their choice.

Box 2.
Acid-base balance
(decompensated metabolic acidosis)
Evaluate the acid-base balance given the following values:
pH 7.52;
[HCO_3^-] 40 mEq/l;
PCO_2 48 mm Hg.

The second part of workshops was devoted to small group discussions (5 - 6 students/group), which were intended to enhance critical thinking, effective communication and collaborative team-work. Within the Physiology workshop each group was assigned a case scenario (Box 3) on one of the four previously discussed themes and a few questions were formulated to help them establish a direction for discussions. The groups had 20 - 25 minutes to analyse the case data and prepare an answer, then each group introduced its case to the class and discussions were encouraged by the facilitator. After each group's presentation, the students were expected to discuss, ask questions, debate, compare and argue their conclusions in such a way that they would have reached a consensus. A major aim of these sessions was to provide a framework for student-centred learning, with the teacher assuming merely the role of guiding the process. Teachers also set the rules and expectations at the beginning of small group discussions, clarified concepts, encouraged active participation of all group members and summarized the conclusions at the end of sessions.

Box 3.
Case scenario – Kidney function
Evaluate functional status of kidney in a 65-year-old female patient suffering of diabetes mellitus for 15 years who complaints of leg edema, nocturia and hematuria. Her blood pressure is 180/100 mm Hg.
Blood tests: urea 80 mg/dl, creatinine 2.5 mg/dl, glucose 240 mg/dl.
Urinalysis: diuresis 3000 ml/day, density 1010 g/cm^3, pH 4.5, proteins (+++), blood glucose (++), ketones (+), bilirubin (-), urobilinogen (+).
Urine sediment test: 6-8 WBC/per field, 18-20 RBC/per field, 8-10 RBC casts/per field.
Points to consider:
- Urinalysis parameters indicative for kidney function.
- What suggest the values of diuresis, urine density and pH in the case?
- Correlation of urinalysis with other data.

In the last day of the module, students were asked to recall the first case scenario (Box 1) and to make a diagnosis using and integrating knowledge acquired throughout the course. The module ended with a session of joint teaching, intended to correlate knowledge across all disciplines involved. *The multidisciplinary approach of renal patient* has incorporated content from basic, paraclinical and clinical disciplines focused on the process of integrating data to achieve a high level of confidence when making a diagnosis.

Student selection

A total number of 66 students participated, 35 in 2013 and31 in 2015. Students were selected based on letters of intent and interviews. In their letters of intent, the students were asked to explain why they consider useful to attend the course. Not surprisingly, most of the students declared their main aim was to acquire new medical information in general or specifically regarding the urinary system, although some 30% of them also mentioned the integrative perspective would help them become better doctors, with some also stressing the importance of the modular course for their personal and professional development. The interviews were based on a set of five questions, with each answer being awarded 1 to 5 points by two interviewers individually and the overall score was used to establish a hierarchy at the end of the interviews. It was considered this approach will help ensure both transparency and equity. Although these questions were previously prepared and asked, the interviewers focused on the discussions raised and not merely on the simple answers of students.

RESULTS

At the end of the course, students' knowledge was assessed by a multiple choice question (MCQ) test, while the modular course itself was evaluated by students through questionnaires. The overall results showed students were able to discriminate between normal and pathological results and to correlate clinical presentation with results. They also acquired a better understanding of the newest developments in the field. By developing and implementing the module, teachers gained experience in working together, as they had to harmonize course contents in order to avoid repetition or resolve discrepancies.

Student assessment

Each teacher provided 10 MCQs, out of which 50 were selected for the final test, with 20 discipline-based questions and 30 integrated questions. Overall, MCQ results were above average, which was expected since this was a free chosen activity by students who were among the first 10% of their respective year of study. All the students scored over 65%.

Module evaluation

A questionnaire for students (Box 4) with 8 closed and 2 open questions was formulated to evaluate the modular course and to get their feedback. A five-level Likert scale

was used (where 1= strongly disagree and 5=strongly agree) for the close-end questions. Analysis of student questionnaires (Figures 1 and 2) showed a high level of satisfaction (average score range: 4.31 – 4.73 in 2013 session and 4.5 – 4.96 in 2015).

Box 4
Questionnaire for students

1. Lectures answered my needs and expectations.
2. Workshops helped me understand theoretical concepts taught during lectures.
3. Teaching methods were interactive.
4. Lecture materials were well structured.
5. Labs were adequately equipped.
6. This module increased my interest in the subject.
7. This module increased my knowledge of urinary system.
8. Adequate methods were used for student assessment, which mirrors my knowledge.

The two open questions were intended to give students the opportunity to comment on the strengths and weaknesses of the module as perceived by them. They were instructed to address the modular course as a whole, as well as any other particular aspects they might considered relevant, including but not limiting to those evaluated in the closed questions. Most students appreciated the better level of interaction with teachers, particularly during the small group discussions; the integration of basic sciences (including Physiology) with clinical scenarios is facilitating learning and comprehension; the integrated approach of medical education is better than the traditional one. Among the weaknesses of the module, students pointed out that too much and sometimes unnecessary theoretical information was delivered. They found the integrated approach more challenging, although hard to pursue at times.

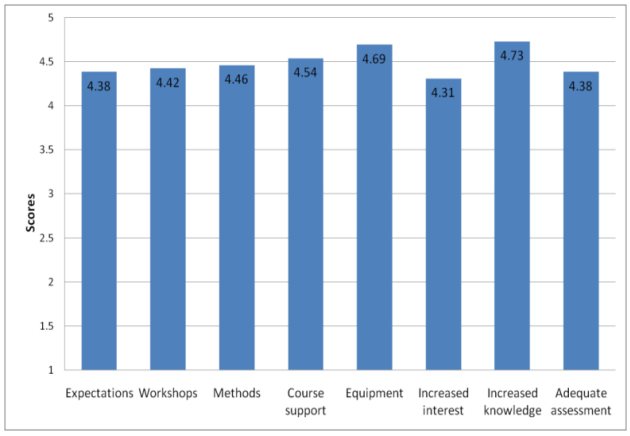

Fig. 1. Module evaluation. Urinary System - 2013

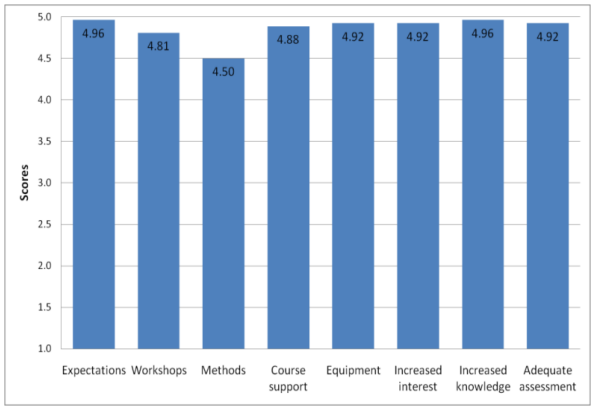

Fig. 2. Module evaluation. Urinary System - 2015

DISCUSSION AND CONCLUSIONS

During the last decades, medical education throughout the world is undergoing major changes, since the need for new, fresh paradigms was recognised. A shifting point in the views on medical education came with the adoption of Edinburgh Declaration [8] during the World Conference on Medical Education in 1988. The set of proposed actions included building the curriculum to achieve professional competence and social values, ensuring the national health priorities are reflected in the curriculum content and integrating education in practice through a problem-based approach. The declaration triggered an extensive reform of medical education systems around the world, as it was agreed by medical educators in all the regions of the world [9].

The Bologna Declaration [10] in 1999 has furthered the reform within the European Union, this time encompassing the educational process as a whole, regardless of the field of study. It was aimed to increase the competitiveness of the EU higher education, while making national systems more comparable and compatible. The developments since are commonly referred to as *the Bologna Process*. The principle of harmonization involved, among others, the creation of a two-cycle structure, first degree – undergraduate level (three or four years) and master's degree (five years from entry), with the third cycle, doctoral level (seven or eight years since entry) [11], added later. However, medical educators have been concerned that the Bologna process would not be suitable to medicine, especially when it comes to the two-cycle framework, which was understood as a pre-clinical (first cycle)/clinical (second cycle) model [12]. Two projects, MEDINE (2004-2007) and MEDINE2 (2009-2013) involving all the EU member states, promoted by universities and funded by the European Commission, have addressed various aspects of medical education in connection with the Bologna process [13]. A report [14] on common learning outcomes/competences for the Bachelor of Medicine in Europe was issued following the surveys conducted within

the second project, concluding that a fully integrated curriculum with early clinical experience is possible and desired. A European learning outcomes framework based on integrated curricula would facilitate the application of Bologna process to medical education [15] and this clearly shows its principles are able to drive quality improvement and harmonization of medical education throughout Europe.

Romania, as a signatory of the Bologna Declaration, has started a top-down reform of its higher education system, which, however, remains rather technical in nature [16], with implementation of the two-cycle model, the European Credit Transfer System or the quality assurance system. Moreover, as in the case of other European countries, modular study programmes are merely attempted, with less than 25% of elective courses, while the medical sciences programmes are not designed according to the Bologna two-cycle model [17].

Shifting from a traditional to an integrated curriculum requires much effort in order to correlate subjects previously taught in different, often unconnected departments, but there are several advantages that need not to be neglected: lower fragmentation of medical courses, motivation and shaping attitudes of students, higher effectiveness of teaching, higher level objectives, improved staff communication and collaboration and rationalization of teaching resources [6].

This module has shown the efforts to integrate pre-clinical, paraclinical and clinical knowledge using student-centred teaching strategies with problem-based learning as central approach are very useful for fine tuning both teaching and learning. Three major conclusions can be drawn, as follows: (1) Small group teaching enhances the quality of teaching and learning processes. (2) Close interaction with students enables appropriate feedback for improvement of teaching process. (3) An integrated approach to teaching and learning allows students to better integrate pre-clinical knowledge into clinical context.

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INTEGRAREA FIZIOLOGIEI ÎN CURSUL MODULAR „SISTEMUL URINAR”

REZUMAT

Universitatea de Medicină şi Farmacie Victor Babeş Timişoara a derulat un proiect care a inclus o serie de cursuri modulare extracuriculare în vederea evaluării impactului generat de o curriculă medical integrată. “Sistemul urinar” a făcut parte din acest demers. Cursul modular a asigurat integrarea verticală şi orizontală, pentru a oferi studenţilor înţelegerea aprofundată a structurii şi funcţiilor renale, precum şi anumite caracteristici ale diferitelor patologii renale şi a fost organizat sub forma a două şcoli de vară (în anii 2013 şi 2015). Modulul, constând în cursuri şi aplicaţii practice, a fost programat pe perioada unei săptămâni şi a inclus workshopuri şi discuţii pe grupuri mici în format PBL (*problem-based learning*). Au fost selectaţi 66 de studenţi în total, pe baza scrisorilor de intenţie şi a interviurilor. La sfârşitul şcolii de vară, cunoştinţele acumulate de studenţi au fost evaluate printr-un test tip MCQ, iar cursul modular a fost evaluat de studenţi printr-un chestionar. Rezultatele studenţilor au fost peste medie, ceea ce era de aşteptat, deoarece a fost vorba de o activitate liber aleasă de studenţi plasaţi între primii 10% din anii lor de studii. La sfârşitul cursului studenţii au fost capabili să facă diferenţa între rezultatele normale şi patologice, să coreleze cazurile clinice cu rezultatele şi au dobândit cunoştinţe mai aprofundate în domeniu. În acelaşi timp profesorii au câştigat experienţă în ce priveşte munca în echipă. Experienţa acumulată în cadrul şcolii de vară a fost unanim apreciată, aşa cum a reieşit din analiza răspunsurilor la chestionarele date studenţilor şi profesorilor. Concluzii: (1) Metoda predării/învățării în grupuri mici îmbunătățește atât calitatea procesului de predare, cât și a celui de învățare. (2) Interacțiunea strânsă cu studenții facilitează obținerea feedback-ului necesar pentru perfecționarea procesului de predare. (3) Abordarea integrată a predării şi învăţării permite studenţilor o mai bună integrare a cunoştinţelor preclinice în contextul clinic.

Cuvinte cheie: educaţie medicală, curs modular integrat, discuţii pe grupuri mici

INTERACTION BETWEEN ALEXITHYMIA AND OBSESSIVE - COMPULSIVE BEHAVIOR TO HEMOPHILIAC PATIENTS

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ABSTRACT

Introduction. Hemophilia is "a genetic transmitted disorder", it belongs to the category of rare diseases, diseases that face devastating and crippling developments, affecting each person beyond physical problems.

Material and methods. The present study is descriptive and analytical and took place within the Medical Center for evaluation and rehabilitation "Cristian Serban" Buziaş and Pediatric Clinic Timisoara, 100 patients that have presented themselves for evaluation of comprehensive treatment and recovery were monitored.

Results. Our results show that the prevalence and levels of alexithymia were higher in patients with low health perspective, with poor social relations and with obsessive-compulsive behaviors.

Conclusions. Psychological consequences of patients experiencing a chronic condition and with complications are numerous: the possibility of disability, dependency of the basic family, emotional and behavioral disorders, the inability to sustain themselves, difficulties of integration into social life.

Key words: haemophilia, alexithymia, obsessive-compulsive behavior

INTRODUCTION

The presence of the malady in the patient's life compels him to deal with the changes imposed on his body for all his life. This event can be devastating both physical and psychical and loads the patient with a series of losses that customize this pathology: loss of independence; lifestyle changes; uncertainties regarding the future; the feeling of helplessness; friends separation; changes in physical health; changes in body image; emotional and behavioral disorders [1].

Aim: Hemophilia form, obsessive-compulsive behaviors level, general health, physical functioning, social relations domain may be predictors of the alexithymia level in hemophiliac patients.

MATERIAL AND METHODS

The batch for clinical study is made up of 100 (50%) patients with hemophilia A (35.5%) and B (14.5%).

By the form of severity there can be found 83 subjects diagnosed with severe hemophilia (41.5%), 13 subjects diagnosed with medium hemophilia (6.5%) and 4 diagnosed with mild hemophilia (2%) (Figure 1).

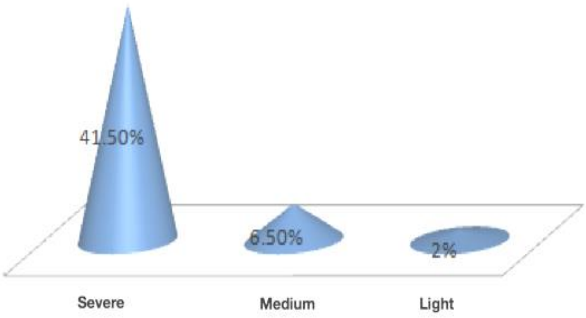

Fig. 1. Distribution by type of hemophilia patients

To verify this hypothesis we have used the results obtained at SECOC (scale for evaluation of obsessive-compulsive Behaviors-Yale-Brown Scale) to measure the level of obsessive-compulsive behaviors and for the measurement of alexithymia results from TAS (Toronto Alexithymia Scale) have been used. For the

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measurements of the dimensions of general health and physical functioning the results of the Questionnaire for health and quality of life (MOS- SF–36) have been used, and to measure the domain social relationships results from World Health Organization Quality of Life (WHOQOL) have been used.

RESULTS AND DISCUSSION

To test the hypothesis a multi-hierarchical linear regression in two steps was made. Given that both the dependent variable and criterion (alexithymia level) as well as predictors are measured by means of a numerical scale, it means that the conditions have been met for the use of linear regression [2]. In step one the criterion form hemophilia was introduced (medium, severe) because this variable to be kept in check, and in step two predictors subjected for analysis were introduced: the form of hemophilia, obsessive-compulsive behaviors level, general health, physical functioning, social relations.

Table. I. Values obtained in the form of haemophilia dimensions, the obsessive compulsive behavior, general health, physical functioning, the social relations and alexithymia

	Lot	Mean	Standard deviation
Form of haemophilia	100	2.79	0.49
Obsesive-compulsive behavior	100	9.67	6.93
General health	100	42.43	17.13
Physical functioning	100	54.75	22.11
Social relationships	100	11.49	1.13
Alexithymia	100	69.83	12.43

Table II. The correlation matrix of variables in subject

	Form of haemophilia	Obsesive-compulsive behavior	General health	Physical functioning	Social relationships
Form of haemophilia	-				
Obsesive-compulsive behavior	.076	-			
General health	-.16	-.03	-		
Physical functioning	-.27	-.18	.52*	-	
Social relationships	-.03	.13	.13	.25*	-

To identify any instances of collinearity, the matrix of correlations have been installed between predictors that were included in the study. According to the data from Table I, in which the matrix of correlations between predictors in the model is presented, a situation of multicolinearity has not been identified. Analyzing Table II, we noted that between hemophilia form, alexithymia, pain, general health, environment, there are not very strong correlations, which indicates that the possibility of a multicolinearity is reduced [2].

Table III. The value of R^2 and R^2 adjusted

Regresion model	R	R^2	R^2 Adjusted
1	**.002**	**.000**	**-.010**
2	**.549**	**.302**	**.264**

Predictors: the form of hemophilia, the level of obsessive-compulsive behaviors, physical functioning, general health, social relations.

Table IV. The value of significance test F

Regresion model 1	**Sum of squares**	**Df**	**Mean of squares**	**F**	**Sig**
Regresion	.083	1	0.83	.001	.982
Waste	15316.027	98	156.289		
Total	15316.110	99			
Regresion model 2	**Sum of squares**	**Df**	**Mean of squares**	**F**	**Sig**
Regresion	4618.473	5	923.695	8.116	.000
Waste	10697.637	94	113.805		
Total	15316.110	99			

As a result of data analysis it is observed that the first model does not explain better than those based on mean the data dispersion, $F(1.98)= .982$, $p> .05$. Model number 2, which comprises in addition to the form of hemophilia, obsessive-compulsive behaviors, physical functioning, general health, social relationships domain, is significantly statistically better than the one which was obtained on the basis of the mean of the values $F(5.94)= 8.116$, $p< .01$. Of the five predictors, three of them (the level of obsessive compulsive behaviors, overall health, and social relations) is presented statistically significant as it can be seen in table 4. This model is able to explain in a ratio of adjusted form 26,4% (R^2 adjusted = .264) of the evolution of dispersal obsessive-compulsive behaviors level, while the remaining 73.6% remains unexplained, because it is due to other factors not entered in the regression model.

Table V. The predictors value included in the regression model

Predictors **Model 2**	Non-standardized coefficients		Standardized coefficients	t	Sig.
	B	Standard error	Beta		
Intercept	109.161	12.904		8.459	.000
Form of hemophilia	-1.675	2.240	-.067	-.748	.457
Obsessive-compulsive behavior	.736	.161	.410	4.579	.000
General health	-.241	.074	-.331	-3.262	.002
Physical functioning	.050	.061	.090	.822	.413
Social relationships	-2.987	.999	-.271	-2.990	.004

Furthermore, Table V gives us important information about those predictors that contribute to the effectiveness of the model as well as about their share within the regression model. On the basis of the significance test t shown in Table V, it is found that from the five predictors included in model three are contributing statistically significant to the evolution of the hemophiliac patients' alexithymia level, i.e. the level of obsessive-compulsive behaviors, overall health, social relations.

The level of obsessive-compulsive behaviors has a positive value (b =.736) therefore establishes a direct relationship between alexithymia and obsessive-compulsive behaviors level. Thus, when the level of obsessive-compulsive behaviors increase a unit, alexithymia tends to increase by one unit (specifically with 0.73) under conditions in which the constant influence of other predictors is kept constant.

Overall health has a negative value (b=.241) therefore sets an inverse relationship between overall health and alexithymia level. Thus, when it increases by one unit, alexithymia level tends to decrease with one unit (more precisely -0.24) under conditions in which the influence of other predictors is kept constant.

The field of social relationships has a negative value (b=-2.987) therefore sets an inverse relationship between the field of social relationships and the alexithymia level. Thus, when it increases by one unit, the alexithymia level tends to decrease with one unit (more precisely-2.98) in the terms on which the influence of other predictors is kept constant.

Other predictors, contrary to initial expectations according to statistical results do not significantly influence the increase or decrease of the level of alexithymia to ailing hemophilia patients.

Our results show that the prevalence and level of alexithymia were higher in patients with low perspective on health, with low social relations and with obsessive-compulsive behaviors.

In regards to the study of the relationship between alexithymia and obsessive-compulsive behaviors, it is found that it is consistent with the results found in other studies, which noted that the percentage of patients with a weak or absent perspective of alexithymia is relevant to patients with obsessive compulsive disorder [3, 4].

In the scientific literature, Matsunaga *et al.* have reported significant correlations in patients with obsessive-compulsive disorder and severe alexithymia [5,6]. The results of the study conducted by Rachman *et al.* [7] are consistent with our hypothesis that obsessive-compulsive behavior is predictor for alexithymia, meaning that the greater the severity of obsessions and compulsions increase, the higher is the level of alexithymia.

In another study, a number of subjects 5418 aged between 30 and 97 years were investigated with the TAS-20 regarding the level of alexithymia and the perception of quality of life with the HRQOL. The results were in line with the present study, which included significant correlations between the scales of quality of life and alexithymia [8]. Quality of life depends largely on the ability of hemophiliacs to adapt to chronic disease and to the difficult circumstances which may arise.

Health is a fundamental resource for individuals, communities and societies overall. For the individual, to enjoy a good health is of paramount importance [9].

Lumley *et al.* examined pairing alexithymia with relationships and social skills, with physical and mental health problems. Relations between alexithymia, measured with the TAS-20, social variables, physical health and depression were examined both in young healthy adults and patients.

Alexithymia was associated statistically significant with a deficit of social skills and social relationships. It has been noted the association between alexithymia, somatizations, depression and physical health, and the social support was not a significant one [10]. Terry has conducted a survey on a sample of 158 subjects and they were questioned about the relationship between alexithymia and satisfaction in intimate relationships: the general satisfaction of the relationship and sexual satisfaction in the relationship. The analysis showed a negative relationship between the moderate level of alexithymia and both of the measured variables. These results assist other previous studies showing a strong connection between alexithymia and a range of interpersonal problems [11].

Alexithymia, defined as the inability to identify or describe emotions, has been shown to be associated with a large number of the patient's chronic pain. A number of 129 patients with muscular dystrophy and chronic pain were administered TAS-20, SNR (pain intensity), the

interference of pain (Short Pain Inventory), the SF-36 (scales of mental health and vitality). Significantly elevated TAS scores were significantly associated with the intensity and the interference of pain, and, in the case of vitality and mental health, associations have been reduced [12]. To investigate the relationship between alexithymia and pain in a sample of patients with chronic pain, 100 (67 women, 33 men) patients with chronic musculoskeletal pain were questioned in this regard by completing the TAS-20, PANAS, and VAS. For instance, it was observed that there is a direct relationship between the two, so the pain increases the effect of alexithymia [13].

Roghaye *et al.* investigated, within a research, the role of moderator of self-efficacy in the relationship between alexithymia and pain (pain severity and disability) in patients with chronic pain. 100 patients with chronic musculoskeletal pain, both women and men, participated in this research. All participants were asked to complete the measurement scale of alexithymia (TAS-20), self-efficacy questionnaire (CPSEQ) scale for severity of pain (VAS), and disability questionnaire (PDQ). The results showed that the self-efficacy has lowered the effect of alexithymia in pain and disability severity level. So that it can be concluded that the relationship between alexithymia and pain in patients with chronic pain was influenced by self-efficacy [14].

CONCLUSIONS

Between the level of obsessive compulsive behaviors and overall health it is established a direct relationship, in the sense that the more careful, more precautions by avoiding knocks and falls, the protection related to the choice of clothing, the more they assess their overall health status as being better (adjusted $R^2 = 264$).

- The level of obsessive-compulsive behaviors has a positive value (b =.736) therefore it is established a direct relationship between alexithymia and the level of obsessive-compulsive behaviors.
- Overall health has a negative value (b = -.241), and thus, an inverse relationship exists between the level of overall health and alexithymia.
- The field of social relationships has a negative value (b = -2.987), obtaining an inverse relation between alexithymia and social relations level.
- The higher the level of obsessive-compulsive behaviors increases, the more their need for social support is greater. Social support explores how the person felt, engagement, encouragement support from family and friends.

The relation between the functional physical scale and obsessive-compulsive behaviors is a reversed one, which means that daily activity significantly lowers while the level of protective, reassurance behaviors is increased.

Other predictors, contrary to initial expectations, and according to statistical results do not significantly influence the increase or decrease in the level of alexithymia in patients suffering from hemophilia.

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INTERACȚIUNEA DINTRE ALEXITIMIE ȘI COMPORTAMENTUL OBSESIV-COMPULSIV LA PACIENȚII CU HEMOFILIE

REZUMAT

Introducere. Hemofilia este „o dezordine genetică transmisă", face parte din categoria bolilor rare, boli care se confruntă cu evoluţii devastatoare şi handicapante, afectand fiecare persoană dincolo de problemele fizice.

Material și metoda. Studiul de faţă este unul descriptiv şi analitic şi s-a desfăşurat în cadrul Centrului Medical de Evaluare şi Recuperare „Cristian Şerban" Buziaş şi a Clinicii de Pediatrie Timişoara, au fost urmăriţi 100 de pacienţi ce s-au prezentat în vederea evaluării comprehensive, a tratamentului şi recuperării fizice.

Rezultate. Rezultatele noastre arată că prevalenţa şi nivelul alexitimiei au fost mai mari la pacienţii cu perspectivă scăzută asupra stării de sănătate, cu relaţii sociale scăzute şi cu comportamente obsesiv-compulsive.

Concluzii. Consecinţele psihologice ale pacienţiilor care se confruntă cu o afecţiune cronică şi cu complicaţii sunt numeroase: posibilitatea apriţiei invalidităţii, dependenţa de familia de bază, tulburari emotionale si de comportament, incapacitatea de a se întreţine singuri, dificultăţi de integrare în viaţa socială.

Cuvinte cheie: hemofilie, alexitimie, comportament obsesiv-compulsiv

THE UTILITY OF RING FINGER MEDIAN-ULNAR SENSORY LATENCY DIFFERENCE IN THE EARLY DIAGNOSIS OF CARPAL TUNNEL SYNDROME

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ABSTRACT

Study highlights: Ring finger media-ulnar sensory latency difference is useful in the early diagnosis of carpal tunnel syndrome; Ring finger media-ulnar sensory latency difference can be used as a screening test for carpal tunnel syndrome; Ring finger median-ulnar sensory latency difference can be used as complimentary test for doubtful cases of carpal tunnel syndrome

Objectives: to study the utility of ring finger median-ulnar sensory latency difference in the early diagnosis and assessment of carpal tunnel syndrome.

Methods: 180 patients with 300 hands matched to 100 healthy volunteers (200 hands). Patients where suffering from signs and symptoms of carpal tunnel syndrome and were tested by ordinary sensory & motor NCS of median & ulnar nerves. Patients with negative results were evaluated by ring finger median-ulnar sensory latency difference.

Results: 137 patients (220 hands) showed a difference between median & ulnar nerves sensory responses recording ring finger greater than 0.4 micro second and were categorized to have early mild carpal tunnel syndrome while the remaining patients and control show less or no difference.

Conclusion: the use of ring finger median-ulnar sensory latency difference is helpful in the assessment and early diagnosis of median nerve entrapment in the carpal tunnel because it increase the diagnostic sensitivity and should be used as complementary part of routine electrodiagnostic assessment of carpal tunnel syndrome.

Significance: ring finger median-ulnar sensory latency difference is easy to perform and need no additional equipment, accessories and experience and so it can be useful for screening and aid in the early diagnosis of carpal tunnel syndrome.

Keywords: carpal tunnel syndrome, ring finger median-ulnar sensory latency difference, electrodiagnosis of carpal tunnel syndrome.

INTRODUCTION

Carpal tunnel syndrome (CTS) is a common peripheral entrapment neuropathy which has been related to occupational factors including deviated wrist postures, especially when combined with repetitive and forceful use of the hand [1]. Patients complain of nocturnal numbness and pain in the hand and lateral three digits, and sometimes weakness and disuse of the thumb due to chronic median nerve compression at the wrist [2].

Nerve conduction studies (NCS) are commonly used to confirm the clinical diagnosis of CTS and it has a high degree of sensitivity (95%) and specificity (98%) [3]. However, it had shown that there is no relationship between nerve conduction results and clinical outcome measures after carpal tunnel release [4]. Also correlation of electrodiagnostic severity and clinical severity does not always reveal an exact match although they are highly correlated for diagnosis [5]. In spite of this, NCS provides the most objective, quantitative & non-invasive assessment of myelinated nerve fibre dysfunction, and has an important complimentary function in cases of atypical clinical presentation, or when other underlying causes such as neuropathy are suspected [2].

MATERIALS AND METHODS

This case control study was conducted at the period of July 2012 to May 2015. It included 180 patients and 100 control subjects. Control subjects have the same age and gender parameters as those of patients.

Diagnosis of CTS was based on the American Academy of Neurology clinical diagnostic criteria [6]

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summarized here: paresthesia, pain, swelling, weakness or clumsiness of the hand provoked or worsened by sleep, sustained hand or arm position, repetitive action of the hand or wrist that is mitigated by changing posture or by shaking of the hand; sensory deficits in the median innervated region of the hand and motor deficit or hypotrophy of the median innervated thenar muscle; symptoms elicited by Phalen test (1 min passive forced flexion of the wrist), performed on each patient.

Exclusion criteria

1. positive ordinary NCS
2. clinical or electrodiagnostic features of polyneuropathy or radiculopathy
3. paste history of disease that affect nerve function like diabetes
4. drug or toxic exposure that damage nerves like chemotherapeutic agents

Every patient undergoes sensory NCS of median nerve recording at index finger and ulnar nerve recording at the fifth finger. Also motor NCS was done for median and ulnar nerves with F-wave studies.

Patients with negative results on these tests were included in the study. They underwent sensory NCS for median and ulnar nerves recording ring finger and stimulating at the wrist at equal distances.

An onset latency difference of 0.4 μsec was considered as abnormal and supports the diagnosis of CTS.

Statistics

The collected data were revised and introduced to a computer using Statistical package for Social Science (SPSS 15.0.1 for windows; SPSS Inc, Chicago, IL, 2001). Data were presented and suitable analysis was done according to the type of data obtained for each parameter. Data were expressed as Mean ± SD for quantitative measures and both number and percentage for categorized data. The comparison between two groups was assessed using the unpaired t-test for numeric variables. The comparison between three groups was assessed using ANOVA test for parametric data. ROC curve was used to find sensitivity and specificity of the test (7).

RESULTS

Demographic data

The demographic data of patients and controls is shown on Table I.

Table I. Demographic data of study group.

Variable	Patient	Control
Age (years)	36±10	30±8
Sex (female/male)	162/18	91/9
Duration of symptoms (months)	5	0

Symptom profile

The study found that most patients were presented with parasthesia of hand at median nerve distribution and pain, while control group was symptom free. The symptom profile of patients is shown in Table II.

Table II. Symptom profile of the patients.

Symptom	No. of patients (percentage)
Parasthesia	173 (95%)
Pain	126 (79%)
Nocturnal symptoms	73 (27%)
Weakness	13 (2.5%)

Nerve conduction study findings

The results of nerve conduction study of both median and ulnar nerves both motor and sensory findings are shown in Tables III, IV, and V.

Table III. NCS findings of median nerve motor study for both patients and control.

Variables			P-value
Latency (ms)	**Patient**	3.3±0.4	0.003
	Control	2.8±0.1	
Amplitude (mv)	**Patient**	9.7±2.1	0.004
	Control	7.7±1.4	
CV (m/s)	**Patient**	58±5	0.8
	Control	58±6	

Table IV. NCS results of ulnar nerve motor study for both patients and control.

Variables			P-value
Latency (ms)	**Patient**	2±0.3	0.9
	Control	2±0.2	
Amplitude (mv)	**Patient**	10.7±1.8	0.3
	Control	9.9±1.6	
CV (m/s)	**Patient**	61±5	0.8
	Control	61±6	

Table V. NCS findings of median nerve sensory study for both patients and control.

Variables			P-value
Latency (μs)	**Patient**	2±0.3	0.9
	Control	2±0.2	
Amplitude (μv)	**Patient**	10.7±1.8	0.3
	Control	9.9±1.6	

Table VI. NCS findings of ulnar nerve sensory part for both patients and control.

Variables			P-value
Latency (μs)	**Patient**	1.8±0.2	0.5
	Control	1.7±0.1	
Amplitude (μv)	**Patient**	42±20	0.5
	Control	37±12	

Table VII. The results ring finger median-ulnar sensory latency difference in both patients and control.

	Patient	Control	P-value	Sensitivity	Specificity
Latency difference (0.4μs)	0.7±0.5	0.2±0.1	0.002	70%	100%

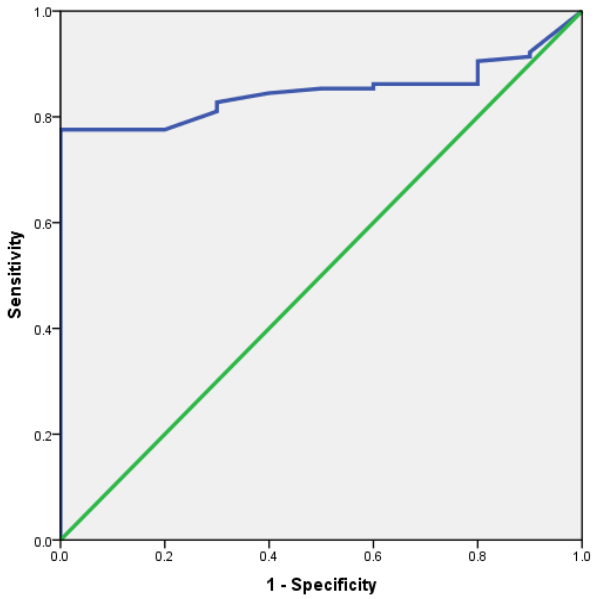

Fig. 1. ROC curve showing sensitivity and specificity of ring finger media-ulnar sensory latency difference.

DISCUSSION

The most commonly reported symptom is parasthesia and this is rational because most of patients presented in the early phase of disease when sensory fibers only or more severely affected by compression than motor fibers. Pain is the second most common presenting symptom and it might reflect both motor and sensory fiber lesion [8].

Motor weakness was the last commonly reported symptom indicting that motor fibers are less severely injured.

The diagnosis of carpal tunnel syndrome is difficult and complicated by the lack of agreement on a "gold standard" diagnostic method for ensuring its presence or absence [9], however a rigorous diagnosis of CTS is needed because it forms the basis of accurate treatment. Poor response or outcome of surgical treatment is often related to poor initial diagnosis [8].

There is a diversity of electrophysiological techniques that are used to assess median nerve conduction across carpal tunnel. These tests are the routine motor and sensory conduction studies of the median nerve which has a sensitivity of 49-84%. Unfortunately, these electrophysiological tests can be normal in a considerable number of patients with CTS. The use of other more sensitive comparative techniques that compare the median sensory or motor conduction across carpal tunnel with an adjacent nerve in the same hand which does not pass through the carpal tunnel and presumed to be normal (direct internal comparison) [10]. These comparative tests increase the sensitivity of electrodiagnosis of CTS to 95% [11]. In addition to that, comparative tests help to eliminate the effects of temperature, age and even of superimposed diseases on the results. In this circumstance, the presence of at least two abnormal sensitive electrophysiological techniques for the diagnosis of CTS is required [12].

The commonest reference nerve is the ulnar nerve. It is well known that ring finger has dual sensory innervation with its lateral aspect supplied by median nerve while the medial aspect supplied by ulnar nerve. The median sensory innervation to ring finger is considered to be highly susceptible to impairment due to transverse ligament compression in the wrist. Monga and Laidlow (1982) [13] found that the amplitude of the sensory nerve action potentials of the ring finger always were lower than those of the index and middle finger, even in healthy subjects. This fact indicates that sensory innervation for the ring finger is provided by a lesser number of fibers from the median nerve than is the case for the index finger and the middle finger; hence its threshold for injury is lower. Monga and Laidlow (1982) [13] and Uncini *et al.,* 1989 [14] reported that the sensory median fibers of the ring finger are damaged earlier than those of the index finger. The reason is possibly the anatomic location of the sensory fibers in the median nerve below the transverse ligament, so that the sections supplying the ring finger are exposed to compression especially early.

Because of all that, the median versus ulnar sensory latency difference recording ring finger is one of the comparative tests with high sensitivity and specificity in the diagnosis of CTS [15].

Uncini *et al*. [14] reported that median versus ulnar digit four sensory latency difference test had the highest sensitivity in comparison to other comparative tests. Also Celik and Guven reported that median sensory CV of the digit two had sensitivity of 81.5% while median versus ulnar digit four sensory latency difference had a sensitivity of 92% [16].

Other comparative tests possibly have lower sensitivity like 2nd lumberical interosseus latency difference motor study (83%) and median-radial sensory study (82%) [17].

The current study found that the sensitivity of this test was 70% and the specificity was 100%. Report from other studies give widely variable results like Andary (42%) [18], Jackson & Clifford (44%) [19], Uncini *et al.* (78%) [20], Pease *et al*. (88.6%) [21], Monga *et al*. (93%) [22], Cioni *et al.* (99.2%) [23], Charles *et al.* (100%) [24] and

Johnson *et al.* (100%) [25]. The possible explanation for the variability between our results and the above results is our patients have mild CTS and showed normal routine NCS of median nerve. So if we select patients with CTS with variable severities, the sensitivity would be higher and might reach up to 95-100%. Other causes of controversy in the results could be due to selecting patients: is there difference in age group, are the symptomatic hands due to CTS? Is there nerve compression with structural lesion or just ischemic reversible initial abnormalities? Are there other causes? Is the "routine" median sensory nerve conduction normal or near normal [26]?

The study has few limitations like the absence of gold standard test for the diagnosis of CTS. Also having a larger sample size of patients with mild CTS in whom the routine tests is normal will help to confirm the results.

CONCLUSION

Ring finger median-ulnar sensory latency difference is a reliable method for the screening and early diagnosis of CTS. It is performed easily and quickly without additional equipment or accessories and give high sensitivity and specificity in the diagnosis of CTS.

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RH ISOIMMUNISATION IN NEONATES: RISK FACTORS, INCIDENCE, COMPLICATIONS AND TREATMENT

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ABSTRACT
Rh incompatibility is a hemolytic disease of neonates, which is the leading cause for moderate and severe Jaundice and Anemia in fetus and newborn. During pregnancy Rh negative phenotype women may develop antibodies against their Rh positive fetuses RBCs. Rh Disease remains significant problem in obstetric and neonatology, which may lead to severe hemolysis and even hydrops fetalis in fetus and newborn without proper and timely prevention and treatment.
Keywords: Rh isoimmunisation, neonates, risk factors, jaundice.

INTRODUCTION

Hemolytic Disease of the fetus and newborn can result from many blood groups incompatibility, but the Rh Isoimmunisation is responsible for the most of moderate and severe cases despite the widespread use of anti D immunoglobulin as prevention method [6]. The hemolytic disease in infants and newborns is an immune mediated destruction of fetal and newborn RBCs. This is usually due to antibodies made by the mother directed against the fetus red blood cells. It is typically caused by Rh incompatibility, which occurs when there is difference between the Rh blood group of the mother and the fetus. Typically happens when the fetal red blood cells express a paternally inherited red blood cell antigen (Rh positive) which is not present on maternal red blood cells (Rh negative) [9].

The objectives of this paper is to study the prevalence of Rh negative phenotype, incidence of Rh disease, It's risk factors ,and the effectiveness of protective measures and treatment.

METHODS

A prospective study of neonates that have been delivered in BEGA hospital in Timisoara city, Romania for one year period. The period of study was from the first of January 2015 until the end of December 2015. The Data has been collected regarding The Blood Rh and ABO group types for both the mother and newborn, Gestational Age, Gravidity and Parity numbers, abortion history, type of delivery, family history of Jaundice, pathologies during pregnancy and the method of treatment.

If the Newborns have developed clinical findings suggesting newborn hemolytic disease, the total serum bilirubin and hemoglobin levels were measured.

If Plasma Bilirubin >4mg/dl and/or hemoglobin level less than 15mg/dl for full term neonate and less than 13 for preterm neonates, then the newborns were admitted and full investigations and treatment started.

If the clinical findings has not been suggestive for hemolytic disease, and the Bilirubin blood levels were below 4 mg/dl and Hemoglobin levels were above 15 for full term and above 13 for preterm newborns then the newborns were discharged home and the family has been informed to bring the newborn after 2-3 days for follow up.

Investigations done for neonates:

1) Blood group and Rh typing
2) Total Serum Bilirubin (TSB)
3) Hemoglobin
4) Hematocrit
5) APGAR score

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6) Gestational Age
7) Other Pathologies

Investigations done for mothers:
1) Blood group and Rh typing
2) Gravidity and Parity numbers
3) Abortion History
4) Pathologies during pregnancy
5) Type of Delivery

RESULTS

2456 Pregnant women delivered during this period, all of them have been examined for their Rh type, 186 of them have Rh negative. From those 186 pregnant women 167 delivered neonates whom Rh is positive, and 19 whom Rh is negative. Those 19 Rh negative newborns were excluded from the study (because only Rh positive neonates can develop Rh disease).

Rh phenotype prevalence: the prevalence of preganant women whom Rh phenotype is negative in BEGA hospital during one year study has been determined from the data the have been collected.

Out of 2456 pregnant women there are only 186 whom Rh phenotype is negative [7.5%] .

The National Center for Biotechnology Information (US) has conducted a study in 2005 about the Rh negative phenotype prevalence regarding the races. They have found the Rh negative phenotype most commonly present in the Basques race (30-35%) then Caucasians (15%), less common in Blacks (8%), and rare in Asians (1%) [5].

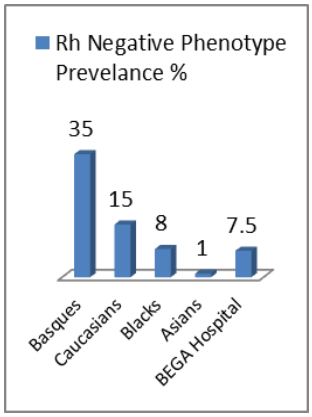

Fig. 1. The prevalence of Rh (-) phenotype around the world comparing to our study

Incidence: the incidence of Rh disease in BEGA hospital has been deducted from the data in our study, out of 2456 pregnant women, there are 186 women with Rh negative, 167 whom newborns have Rh positive phenotype, and only 24 newborns developed the Rh disease. Thus the incidence of Rh disease in BEGA hospital during one year study, from first of January 2015 till the end of December 2015 is 24 out of 2456 (1%).

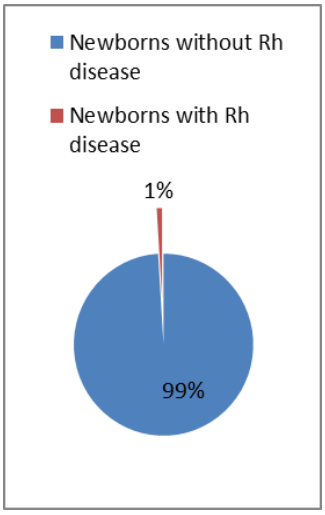

Fig. 2. The incidence of Rh disease in BEGA Hospital

The incidence of Rh disease in population depends on the prevalence of Rh negative phenotype and on the prenatal care. The incidence of Rh disease markedly reduced since blood Rh typing and the administration of anti-D immunoglobulin became a routine prophylaxis measure. In the US, the Centers for Disease Control and Prevention reported in 2003 the incidence of Rh hemolytic disease is 6.8 cases per 1000 live births [10].

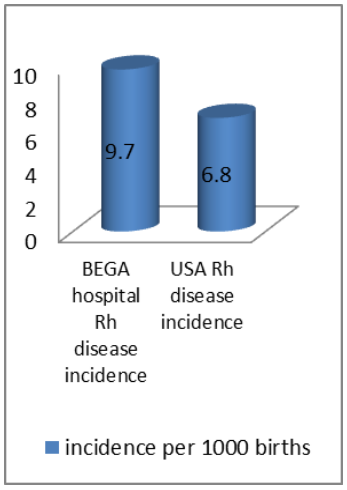

Fig. 3. The incidence of Rh disease in USA comparable to BEGA Hospital

Jaundice etiology: From the 186 pregnant women with Rh negative phenotype, there are 167 neonates having Rh positive phenotype, and only 24 out of those 167 developed hemolytic disease due to Rh incompatibility, the other 143 either have not developed jaundice or developed jaundice due to other causes than Rh incompatibility.

Out of those 143 , 105 have not developed jaundice, and 38 developed jaundice due to variety of casues [18 due to physilogical jaundice, 12 due to obstetrical trauma, 2 due to ABO incompatibility, 6 due to maternal fetal infection].

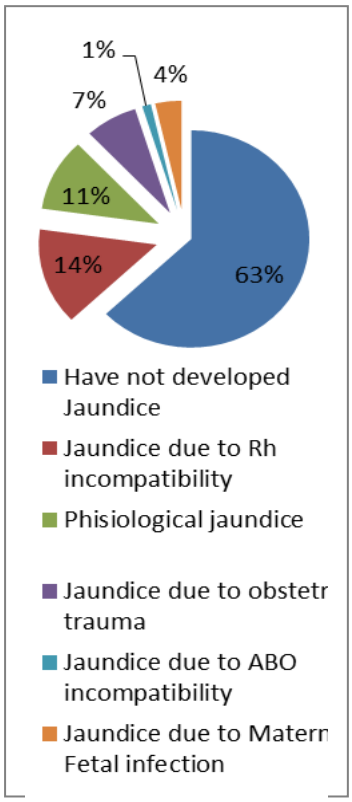

Fig. 4. The distribution of jaundice causes in newborns with Rh+ that have born to Rh negative mother.

Mothers ABO Blood group Distribution: The distribution of ABO blood group type of the mothers with Rh negative phenotype whom newborns have Rh positive phenotype as follow, out of 167 women there are [83 have type **A**, 42 have type **O**, 28 have type **B** and 14 have type **AB**].

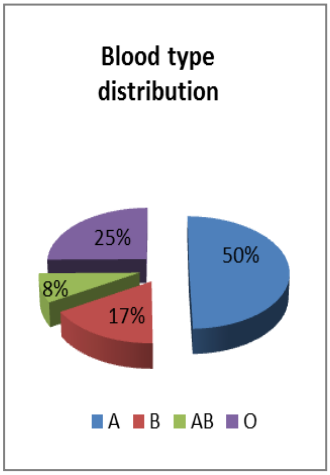

Fig. 5. Distribution of mother's ABO blood group in Rh- mothers

The concurrence of mothers and newborns with the same ABO type has been calculated from the data as the follow: 98 of cases the mothers and neonates have the same blood ABO type and 69 have different blood groups.

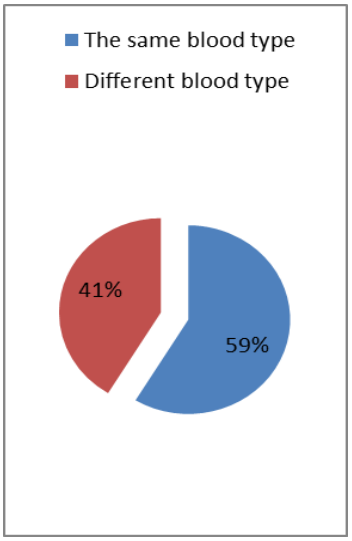

Fig. 6. The concurrence of mothers and newborns with the same ABO

Rh Coexistent with ABO Incompatibility: The prevalence of newborns with ABO incompatibility coexistent Rh incompatibility has been calculated from the data of our study, there are 12 cases out of 167 Rh negative mothers (7%), where the mothers have blood type O and the neonates with either blood type A or B.

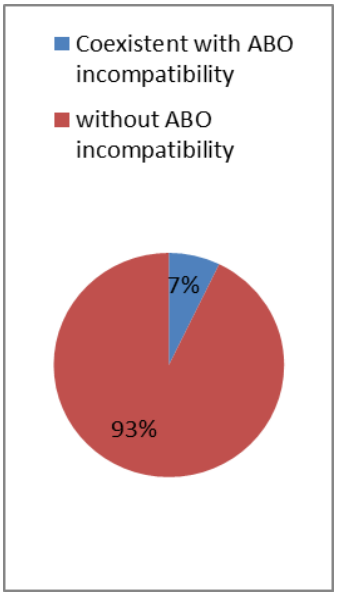

Fig. 7. The distribution of Rh incompatibility Coexistent with ABO incompatibility.

Rh coexistent ABO incompatibility for either the A or B blood group antigen will reduce the risk of maternal Rh sensitization. The rapid immune clearance of the fetal erythrocytes before Rh sensitization can proceed to a significant extent due to ABO incompatibility. Fortunately, hemolytic disease due ABO incompatibility is less severe than that of Rh isoimmunisation, but it confers no protection once sensitization has occurred.

In the 12 cases with Rh coexistent with ABO incompatibility only 2 cases have developed mild jaundice and anemia, and none of them developed Rh sensitization. The results emphasizes what we have discussed in the first chapter that coexistent ABO incompatibility is a protection factor and reduces the Rh sensitization occurrence.

We classified the newborns into two groups, the first group is the ABO compatible newborns, and the second group is the ABO incompatible newborns.

In the first group, out of 155 compatible newborns 24 developed Rh disease (15.4%), and in the second group zero newborns developed Rh disease (0%).

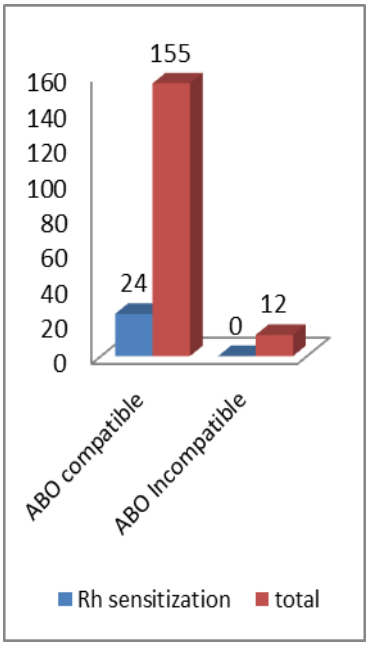

Fig. 8. The distribution of Rh disease in ABO compatible comparing to ABO Incompatible fetuses.

The distribution of Newborns with Rh Incompatibility according to Gestational Age: in our study the gestational age data of 167 neonates has been collected and shown the following number of neonates born in each specific gestational age (41 weeks: 21 neonates, 40 weeks: 49 neonates, 39 weeks: 42 neonates, 38 weeks: 28 neonate, 37 weeks: 8 neonates, 36 weeks: 19 neonates).

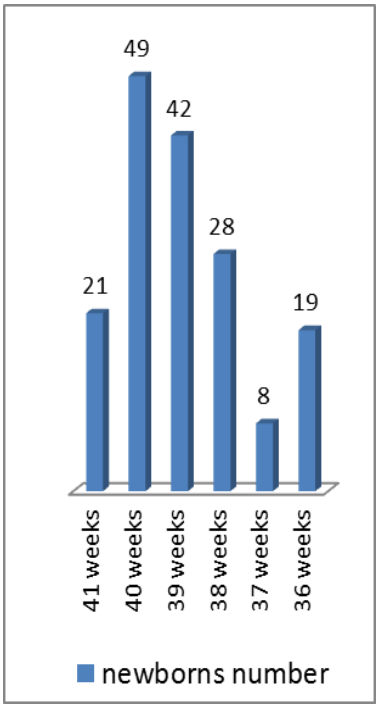

Fig. 9. The distribution of Newborns related to their gestation age

As shown in Figure 10, from 167 newborns, only 19 newborns are premature and 148 are mature. Assuming that newborns who born before 37 weeks are considered premature. The global incidence of preterm delivery was estimated by world health organization to be 9.6% [19]. In our study we can conclude that Rh incompatibility doesn't increase the incidence of preterm delivery.

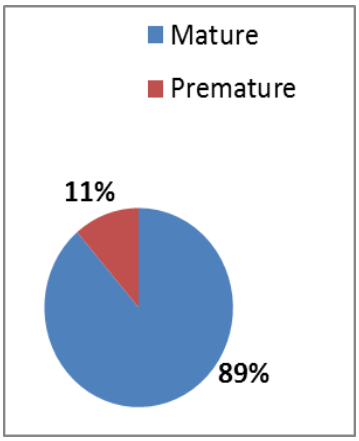

Fig. 10. Distribution of newborns according to maturity.

Distribution of newborns according to Gender: out of 167 newborns, there are 105 male newborns and 62 female newborns. Thus the ratio between male and female is 1.7:1. As shown in Figure 11 there are 63% males which is by far more than female patients.

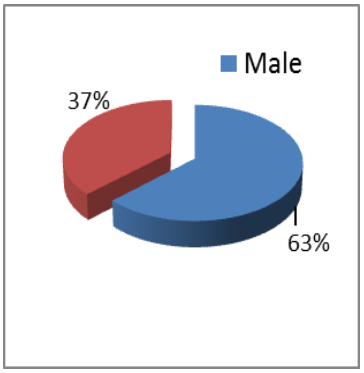

Fig. 11. Distribution of newborns according to gender

We found out that from those who developed Rh disease 20 are males and only 4 females from all the 167 Rh incompatible newborns. As we mentioned in the first part, male infants are reported to have an increased risk to develop Rh disease than females, although the basis for this observation is unclear.

The incidence of Rh disease sensitization in Rh incompatible newborns as follow: 20 patients out of the 105 males (19%), and 4 out of the 62 females (6.4%).

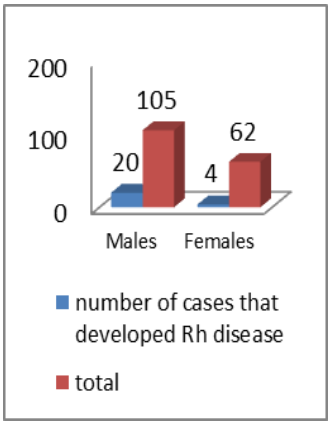

Fig. 12. Number of cases that developed Rh disease according to gender.

The prevalence of newborns who developed anemia in the first day: considering that each newborn who has hemoglobin below 15 mg/dl is anemic we classified the newborns into normal and anemic neonates. We found that 42 out of 169 newborns had anemia in the first day, as it is shown in Figure 13 (25% of the newborns were anemic). Anemia is a common complication of Rh disease which occurs due to hypo-regenerative and hemolytic mechanisms [1,2].

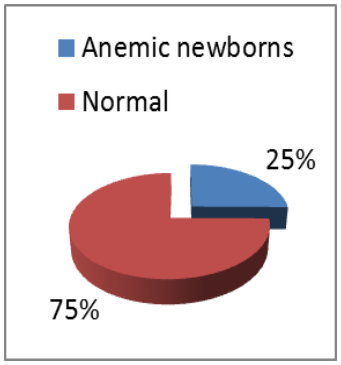

Fig. 13. Prevalence of Newborns that developed anemia in the first day

Distribution of Newborns according to type of delivery: in our study 90 (53.8%) women had delivered by Caesarean section and 77 (46.2%) women delivered natural delivery. From the 24 neonates that developed jaundice due to Rh disease, 16 of them have been delivered by C-section and 8 have been delivered naturally.

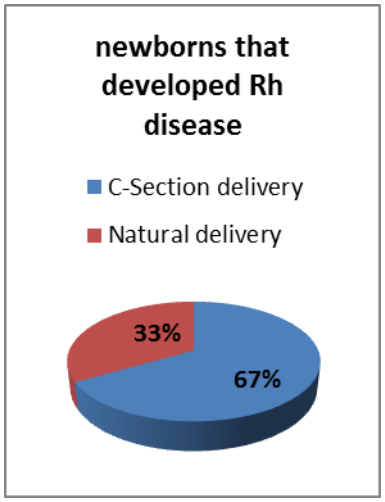

Fig. 14. Development of Rh disease according to type of delivery

We observed that the incidence of Rh disease increased in newborns that have been delivered by C-section.

Distribution of women according to number of pregnancies: in our study out of 167 there are 70 women with I pregnancies, 41 with II pregnancies, 14 with III pregnancies, 7 with IV pregnancies, 21 with V pregnancies, and 14 with VI pregnancies.

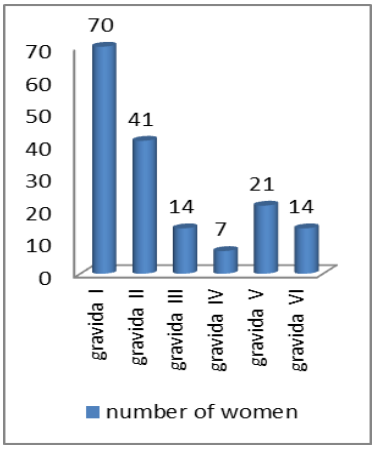

Fig. 15. Distribution of women according to number of pregnancies

We noticed from Figure 15 that there are 70 out of 167 newborns have been delivered in the first pregnancy (Primi-gravida), and 97 are considered multigravida (delivered after more than one pregnancy).

The distribution of Rh disease according to number of gravidity: From the 24 newborns that developed Rh disease there are 4 newborns that are considered gravida I, 6 newborns are gravida II, 3 newborns are gravida III, 2 newborns are gravida IV, 5 newborns are gravida V and 4 newborns are gravida VI.

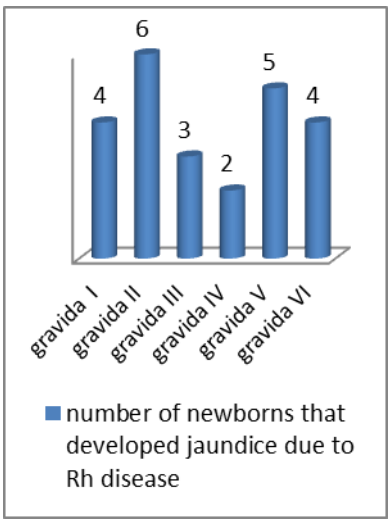

Fig. 16. Shows the number of newborns who developed Rh disease related to the number of gravida.

There are only 4 newborns out of 70 (5%) of primi-gravida mothers developed Rh disease, while there are 20 out of 96 (20.8%) of multigravida mothers developed Rh disease.

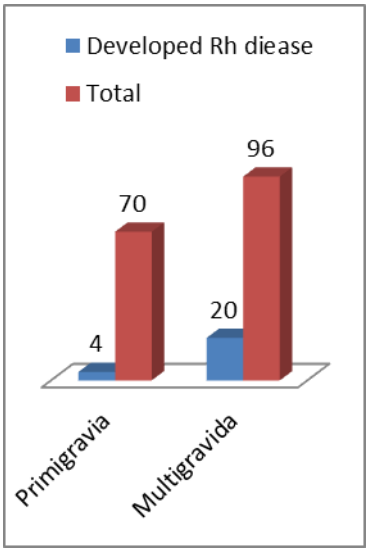

Fig. 17. Comparison between the incidence of Rh disease in primigravida and multigravida.

This result confirms what we have mentioned in the first part, that birth order is significant risk factor, thus the first newborn has minimal risk to develop the disease, unless sensitization has occurred previously. Once sensitization has occurred, subsequent pregnancies are at a progressive risk for fetal and newborn hemolytic disease.

Distribution of cases according to peak Bilirubin levels: In our study we classified the patients in 3 groups according to peak bilirubin levels, first group has bilirubin level below 13 mg/dl, second group between 13 and 16 mg/dl and the third group above 16 mg/dl. In our study we measured the newborns bilirubin levels every day until clear improvement and declining in bilirubin level noticed.

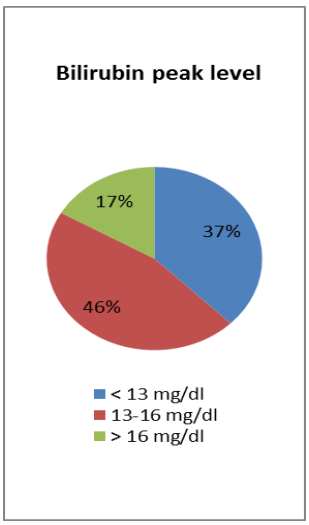

Fig. 18. The distribution of patients according to peak bilirubin level.

We noticed in Figure 18 that most newborns that developed jaundice due to Rh disease had peak plasma Bilirubin Values between 13 and 16 mg/dl.

Treatment: all the 24 newborns with Rh disease developed jaundice in the first 24 hours, 18 of them needed phototherapy alone, 4 patients needed phototherapy and albumin, and 2 patients needed phototherapy, albumin and blood exchange transfusion.

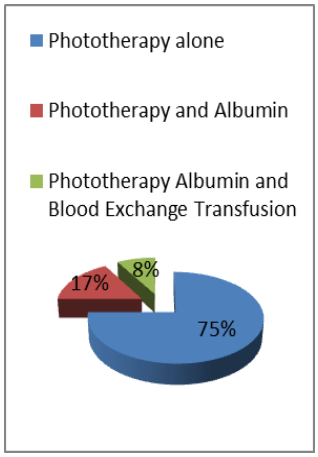

Fig. 19. The distribution of our study group according to the method of treatment

Phototherapy is non-invasive treatment method which decreases bilirubin levels and reduces the number of total exchange transfusions required, thus avoiding neurological and other possible complications of hyper-bilirubin concentration [15,16]. Phototherapy is started if bilirubin rises 0.5 mg/dL/h or if total bilirubin exceeds 10, 12, or 14 mg/dL at 12, 18, or 24 h of life, respectively.

Distribution of cases according to the duration of phototherapy treatment needed: In each day after birth we were measuring bilirubin levels, whenever there is indication for phototherapy we give one episode of phototherapy per day, which is about 6-8 hours duration. In our study from the 24 patient, 7 patients needed 2 days of phototherapy, 11 patient needed 3 days of phototherapy and 6 patients needed 4 days of phototherapy.

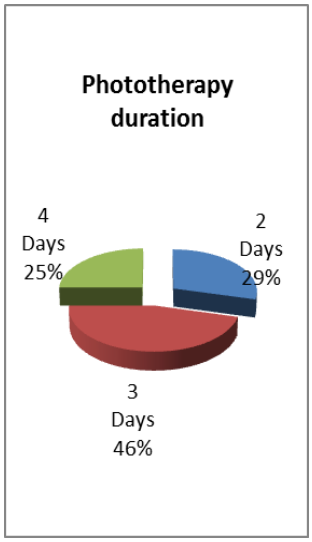

Fig. 20. Show the distribution of patients according to duration of phototherapy

DISCUSSION

Since blood Rh typing and the administration of anti-D immunoglobulins for Rh negative mothers becomes a routine prophylaxis measure, the Incidence of Rh disease has been dramatically reduced.

Despite these advances to prevent the development of Rh disease, the disease still poses major health problem to the mothers and newborns.

The incidence of Rh disease in population depends on the prevalence of Rh negative phenotype, which is varied among the races. The Rh negative phenotype is commonly present in the Basques race about 35% and less common in Asians about 1% [4,7]. Thus it is very important to find the prevalence of Rh negative phenotype in our study in BEGA hospital.

Rh disease still major cause of anemia and fetal hydrops, which resulted from poor prenatal care and prevention, thus in our study we focused on the incidence of Rh disease in BEGA hospital and to compare to the Global results to evaluate the efficacy of the prevention measures and prenatal care.

There are many causes which may lead to jaundice during the neonatal period, thus the differential diagnosis is important to exclude other possible causes than Rh disease. Thus in our study we classified the patients according to their jaundice causes. Many studies and theories claim that ABO incompatibility offers a significant degree of protection against Rh sensitization. The main feature for production of Rh antibodies is the survival of fetal RBCs in the maternal circulation for long enough period to stimulate the anti-D antibodies production. If the fetal RBCs are Incompatible with maternal anti A or B they are rapidly hemolyzed so that the Rh antigens are not available to produce antigenic stimulus [13,14,17].

Therefore in our study we classified the newborns into ABO compatible and incompatible to compare the incidence difference between them. We noticed in some studies that Male infants are reported to have an increased risk of developing the Rh disease, although the basis for this observation is unclear. Thus we aimed to compare the ratio between male and female in our study to observe if there are similar results.

Anemia is considered as common complication of the Rh isoimmunisation, which is caused by the hemolytic mechanism. Thus we obtained the hemoglobin values in the first day of newborns to evaluate the percentage of anemic newborns.

In 90% of cases, sensitization occurs during delivery. Therefore, most first born infants with Rh-positive blood type are not affected because the short period from first exposure of Rh-positive fetal erythrocytes to the birth, which is insufficient to produce a significant maternal IgG antibody response.

The risk and severity of sensitization response increases with each subsequent pregnancy involving a fetus with Rh-positive blood. In women who are prone to Rh incompatibility, the second pregnancy with an Rh-positive fetus often produces a mildly anemic infant, whereas succeeding pregnancies produce more seriously affected infants who ultimately may die in utero from massive antibody-induced hemolytic anemia [11,12,18].

In our study we classified the newborns related to their mother number of gravidity, to compare the incidence of Rh disease in each group to prove that the incidence increased by each subsequent pregnancy as written in theory.

Depending on the severity of the disease, each newborn will develop different degree of jaundice and anemia and so different treatment method will be needed. Some patients needs only phototherapy, others may need albumin and even blood exchange transfusion if severe anemia developed [3,8].

In our study we classified the patients depending on their method if treatment needed, to detect the percentage for each treatment needed and so the severity of the disease.

RESULTS

The prevalence of preganant women whom Rh phenotype is negative in BEGA hospital during one year is Out of 2456 pregnant women there are only 186 whom Rh phenotype is negative [7.5%].

The incidence of Rh disease in BEGA hospital has been deducted from the data in our study, out of 2456 pregnant women, there are 186 women with Rh negative, 167 whom newborns have Rh positive phenotype, and only 24 newborns developed the Rh disease. Thus the incidence of Rh disease in BEGA hospital during one year study, from first of January 2015 till the end of December 2015 is 24 out of 2456 (1%).

From the 186 pregnant women with Rh negative phenotype, 167 neonates has Rh positive phenotype, only 24 out of those 167 had developed hemolytic disease due to Rh Incompatibility (14%), the other 143 either have not developed jaundice or developed jaundice due to other causes than Rh incompatibility. Out of those 143, 105 have not developed Jaundice (63%), and 38 developed jaundice due to variety of casues [18 due to physilogical jaundice (11%), 12 due to obstetrical trauma (7%), 2 due to ABO incompatibility (1%), 6 due to Maternal Fetal infection (4%)].

The Prevalence of Newborns who have Rh Coexistent with ABO Incompatibility has been concluded from the data of our study, there are 12 cases out of 167 Rh negative mothers (7%), where the mothers have blood type O and the neonates with either blood type A or B. We classified the newborns into two groups, the first group is the ABO compatible newborns, and the second group is the ABO incompatible newborns. In the ABO compatible group, out of 155 compatible newborns 24 developed Rh disease (15.4%), and in the ABO Incompatible group zero newborns developed Rh disease (0%).

The incidence of Rh disease sensitization in Rh incompatible newborns as follow: 20 patients out of 105 males (19%), and 4 out of 62 females (6.4%).

The prevalence of newborns who developed anemia in the first day: considering that each newborn who has hemoglobin below 15 mg/dl is anemic we classified the newborns into normal and anemic neonates.

We found that 42 out of 169 newborns had anemia in the first day, as it is shown in figure 13 (25% of the newborns were anemic).

The distribution of newborns that developed Rh disease according to number of gravidity: from the 24 newborns that developed Rh disease there are 4 newborns that are considered gravida I, 6 newborns are gravida II, 3 newborns are gravida III, 2 newborns are gravida IV, 5 newborns are gravida V and 4 newborns are gravida VI.

There are only 4 newborns out of 70 (5%) of primigravida mothers developed Rh disease, while there are 20 out of 96 (20.8%) of multigravida mothers developed Rh disease.

All the 24 newborns with Rh disease developed jaundice in the first 24 hours, 18 (75%) of them needed phototherapy alone, 4 patients (17%) needed phototherapy and albumin and 2 patients (8%) needed phototherapy, albumin and blood exchange transfusion.

CONCLUSION

1. The prevalence of Rh negative phenotype in BEGA hospital is 7.5% which is considered less common than

Basques (35%) and Caucasians (15%) but more than Asians (1%). This means that they are in moderate risk of developing Rh disease comparable to other races.

2. Only 14% of Rh negative women whom fetus has Rh positive antigens develop jaundice due to Rh disease. While 86% either do not develop Rh disease or develop jaundice due to other disease. Thus we deducted that not all Rh negative mother whom fetus is Rh positive develop Rh disease, only 14% of newborns develop the disease.
3. The incidence of Rh disease in BEGA Hospital is 9.9 per 1000 which is similar to the incidence conducted in USA by the US, the Centers for Disease Control and Prevention reported in 2003, 6,8 per 1000. This incidence is much less than two decades ago, and this improvement is due to the blood Rh typing and the administration of anti-D immunoglobulins becomes a routine prophylaxis measure.
4. We concluded from our study that ABO incompatibility offers a significant degree of protection against Rh sensitization since the results showed decreased incidence of Rh disease in ABO Incompatible newborns compared to ABO Compatible newborns.
5. In our study being Male newborns were considered significant risk factor to develop Rh disease, because 20 out of 105 males (19%), and 4 out of 62 females (6.4%) developed Rh disease.
6. Anemia is common complication in Rh disease, since 25% of patients had anemia in the first day of life.
7. Birth order is considered important factor, since there are only 4 newborns out of 70 (5%) of Primi-gravida mothers developed Rh disease, while there are 20 out of 96 (20.8%) of multigravida mothers developed Rh disease. Thus we concluded that multigravida is considered significant risk factor.

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IZOIMUNIZAREA RH LA NOU-NASCUT: FIZIOPATOLOGIE, FACTORI DE RISC, INCIDENTA, COMPLICATII SI TRATAMENT

REZUMAT

Incompatibilitatea Rh este o boala hemolitica la nou-nascut care reprezinta cauza principala de anemie si icter moderat si sever la fetusi si nou-nascuti. In timpul sarcinii femeile cu fenotipul Rh negativ pot dezvolta anticorpi impotriva fetusilor cu Rh pozitiv. Incompatibilitatea Rh ramane o problema semnificativa in obstretica si neonatologie, care poate duce la hemoliza severa si chiar hidrops fetal in viata intrauterina si la nou-nascut fara preventie si tratament adecvat si la timp.

Cuvinte cheie: izoimunizarea Rh, nou-nascut, factori de risc, icter.