Glutathione-S-transferase P1 polymorphism and bronchial asthma in children.

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Abstract:

Glutathione S-tansferase P1 (GSTP1) is abundant in the lung epithelium, plays an important role in cellular protection against oxidative stress and toxic foreign chemicals. It has been suggested that polymorphisms in the GSTP1 gene are associated with bronchial asthma. As significant interindividual and interethnic differences exist in the distribution of xenobiotic-metabolizing enzymes.

Methods:

The present study included 44 atopic asthmatic children during their regular follow up at Al Mounira hospital during the period from January 2008 to June 2009. Their ages ranged between 5- 14 years. Twenty six subjects were males and 18 subjects were females. All cases and control subjects were subjected to detailed history taking for asthma and other atopic manifestations, chest examination, peak expiratory flow rate (PEFR), and laboratory investigations including serum IgE level and genotyping the polymorphisms in the GSTP1 gene using polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) techniques.

Results:

GSTP1 gene polymorphism frequencies for the Val/ Val genotype was detected in (59.1%), in (36.4%) for the Ile/Val genotype and in (4.5%) for the Ile/Ile genotype. While in the control group, it was detected in (66.7%) for the Val/ Val genotype, at (30%) for the Ile/Val genotype and at (3.3%) for the Ile/Ile genotype. We found no statistical significance between asthmatic cases and control group as regard GSTP1 Val/Val, Ile/Val and Ile/Ile in the present study. P value was 0.16. The serum IgE level was significantly higher in asthmatic patients than in control group, P value was 0.000

Conclusion:

These results did not suggest significant association between GSTP1 polymorphism and bronchial asthma in children.

Introduction:

Asthma is a chronic inflammatory disorder of the airways in which oxidative stress in the lungs has been implicated in its pathogenesis (Ercan et al, 2006). This chronic inflammation is associated with bronchial hyper-responsiveness (BHR) that leads to recurrent episodes of wheezing, breathlessness, chest tightness and cough, particularly at night and/or in the early morning. These episodes are usually associated with widespread but variable airflow obstruction that is often reversible either spontaneously or with treatment (Global Initiative for Asthma (GINA), 2008).

The inflammation also causes an associated increase in the existing bronchial hyper-responsiveness to a variety of stimuli (Lemanske et al, 2003).

There is evidence that a genetic predisposition may also alter the capability of the airways to protect itself against inhaled toxic substances from the environment. Several candidate genes were implicated in the development of asthma. It has been reported that prevalence of these candidate genes vary considerably by ethnicity (Ober et al, 2000).

The members of the super-gene family of Glutathione-S Transferases (GST) represent a large and diverse super family of enzymes, with at least 13 GST enzymes belonging to five different families: mu, theta, alpha, pi and gamma. GSTs are known to play an important role in the functioning of anti-oxidant metabolism, in the repairing of damaged ROS and in the detoxification of several xenobiotics such as carcinogens found in tobacco smoking (Hayes and Pulford, 1995). The role played by GSTs may be especially important in response to oxidative stress. (Strange et al, 2000).

The predominant cytosolic GST expressed in the human lung, GSTP1 is a member of the π family, and is located on chromosome 11q13, a hot spot for asthma-related genes (Caroll et al, 2005), and is extensively expressed in fetal lung tissue during a critical stage in lung development (Beckett et al, 1990).

GSTP1, is a candidate gene, because of its role in cellular protection against oxidative stress. Recently, it has been shown that a valine (Val) to isoleucine (Ile) exchange at codon 105 (GSTP1 Val105/Val105) in exon 5 may protect against developing asthma. Although the Val105 variant has higher catalytic efficiency for polycyclic aromatic hydrocarbon diol epoxides, its efficiency for 1-chloro-2, 4-dinitrobenzene is lower compared to the Ile105 variant (Subberg et al,1999).

Genetic polymorphism of the GSTP1 gene, have been known to abolish enzyme activity and increase susceptibility to oxidative stress (Caroll et al, 2005). Previous studies have confirmed or refused an association between either GSTP1 polymorphism and bronchial asthma risk in children.

This study was performed to find out whether the GSTP1 polymorphism is associated with bronchial asthma in children.

Subjects And Methods:

The present study included 44 atopic asthmatic children during their regular follow up at Al Mounira hospital during the period from January 2008 to June 2009. Their ages ranged between 5- 14 years. Twenty six subjects were males and 18 subjects were females.

Patients were classified according to National Asthma Education and Prevention Program (NAEPP) updated (2007). This study included: 15 cases mild persistent asthma, 27 cases moderate persistent asthma, and 2 cases severe asthma.

Thirty healthy subjects with no personal or family history of asthma or other atopic manifestations of matched age and sex were included as a control group.

All cases and control subjects were subjected to detailed history taking for asthma and other atopic manifestations, chest examination, peak expiratory flow rate (PEFR), and laboratory investigations including complete blood picture (CBC) and essay of serum IgE level.

After written informed consents were obtained from the parents of all participants, blood samples were collected from them. Four ml blood was collected in sterile EDTA vacutainers for genotyping and CBC and 4 ml were collected for IgE detection.

Quantitation of IgE in serum:

Quantitative determination of immunoglobulin E (IgE) concentration in human serum was done using a solid phase enzyme- linked immunosorbent assay (ELISA) provided by international immuno-diagnostics, USA.

Genetic study for all cases and control:

GSTP1 genotype was analyzed by polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP).

Genomic DNA was extracted from the whole blood using EZ-10 spin column Blood Genomic DNA Minipreps kit (Biosystems, California, US) and stored at -20°C until genotyping were performed.

DNA amplification (PCR Master Mix):

- PCR Master Mix has been optimized for use in routine PCR reactions for amplifying DNA template in the range of0.2-2Kb.
- PCR Master Mix was provided by Promega corporation, size: 100 reactions, catalogue number # 7502.

Genotypic Primers:

DNA sequences are:

- Sense, ACTGGTGTTGAT-CAGGCGCC.
- Antisense, CCTTCTTGGGTCAGGGTGCAG (Harries et al, 1997).

PCR Amplification:

Genomic DNA (100-150ng) was amplified in a total volume of 25 µl reaction mixture containing reaction buffer of 1.5mM Mg Cl2, 20pmol of each primer, 200µM of each dNTPs and0.5 unit of Taq polymerase.

Initial denaturation was carried out at 94˚C for 5 minutes and then 35 cycles of 94˚C for 1 minute, 58˚C for 1 minute, 72˚C for 1 min, and final polymerization step at 72˚C for 10 minutes. The amplification products were analyzed by gel electrophoresis (2% agarose). The absence of amplification products was consistent with null genotypes. Although this technique does not distinguish between heterozygotes and homozygotes of positive genotypes, it identifies conclusively the null genotypes (Bajpaiet al, 2007).

Genotype Analysis:

Genomic DNA (100ng) was used as a DNA template in a total 50µl volume reaction. The PCR products were diagnosed in 25 µl for 2 hours at 37°Cwith

5 U Abw 261. The digested products were than separated with 3,5% agarose gel stained with ethedium bromide (10 mg/1ml) to visualize the band and to be photographed with gel documentation system. (Harries et al, 1997). figure (1).

Statistical Methods:

- Data was coded and entered using the statistical package SPSS version.
- Data was summarized using mean, SD, and range for quantitative variables.
- P-value less than or equal to0.05 were considered was statistically significant.

Results And Data Analysis:

The present study included 44 atopic asthmatic children, Their ages ranged from 5- 14 years. Twenty eight children (60%) were males and 16 subject (40%) were female. Fifteen (34.1%) cases wetre

classified as mild persistent asthma, 27 (61.4%) cases were Moderate persistent asthma, and 2 (4.5%) cases were severe asthma.

All cases were subjected to IgE quantitation in serum and Genetic study for GSTP1 genotype by polymerase chain reaction (PCR) restriction fragment length polymorphism (RFLP).

The serum IgE level was significantly higher in asthmatic patients than in control group, P value= 0.000.

Table (1) shows comparison of selected data age, sex, asthma severity, serum IgE in asthmatic and control groups.

The serum IgE level was significantly higher in asthmatic patients than in control group, P value= 0.000

Table (1): Characteristics of patient and control groups

		Study Group (N:44)	Control Group (N:30)	P Value
Age/Year	Mean ±SD	8.55± (2.459)	8.53±(2.07)	-
	range	5.08-14.08	5.33±13.08	
Sex	Male	27 (60%)	16 (53%)	-
	Female	18 (40%)	14 (42%)	
Asthma Severity	Mild P	15 (34.1%)	-	-
	Moderate P	27 (61.4%)	-	
	Severe P	2 (4.5%)	-	
Serum IgE (Iu/L)	Mean ±SD	203.45±(143.24)	107.61± (56.09)	0.000
	Range	93-803	19.70-265.70	

Data are represented as frequency (percentage) Significant P value: ≤0.05

As regard GSTP1 gene allelic polymorphism, GSTP1 gene polymorphism Val/ Val genotype was detected in (59.1%), Ile/Val genotype was detected in (36.4%) and the Ile/Ile genotype was detected in (4.5%). While in the control group, it was detected in (66.7%) for the Val/ Val genotype, at (30%) for the Ile/Val genotype and at (3.3%) for the Ile/Ile genotype. P value was not statistically significant.

Data are represented in table (2) and figure (1).

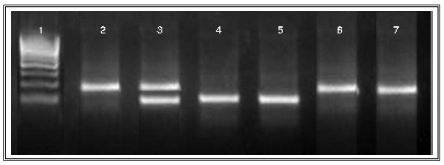
Table (2) Shows comparison between cases and control groups as regard allelic polymorphism:

	Cases	Control	P-Value	OR
P1 Val/Val	26/44 (59.1%)	20/30 (66.7%)	0.801	1.54 (0.57-4.179)
P1 Ile/Val	16/44 (36.4%)	9/30 (30%)		
P1 Ile/Ile	2 (4.5%)	1 (3.3%)		

Data are represented as frequency (percentage).
Significant P value: ≤0.05

P1 Val/Val: Valine\ Valine.
P1 Ile/Val: Isoleucine\ Valine.
P1 Ile/Ile: Isoleucine\ Isoleucine.

Figure(1): Genotyping of GSTP1by PCR-RFLP:



Lane 1: 100 base pair ladder.
Lane 2: the regional band before adding the RE.
Lanes 3: heterozygous case.
Lanes 4& 5: homozygous polymorphic genotype.
Lanes 6& 7: homozygous wild genotype.

As regards asthma severity and allelic polymorphism:

- In cases with mild Peristent asthma 5 cases (19.2%) were Val/Val, 8 cases (50%) were Ile/Val genotype and 2 cases (100%) were Ile/Ile genotype
- In cases with moderate and severe Persistent asthma: 21 cases (80.8%) were Val/Val and 8 cases (50%) were Ile/Val genotype.
- P-value showed no statistical significance. Data are shown in table (3)

Table (3): statistical comparison between asthma severity and allelic polymorphism:

		GSTP1		
	Genotypes	Val/Val	Ile/Val	Ile/Ile
Athma Severity	Mild P	5/266 19.2%	8/16 50%	2/2 100%
	Moderate P And Severe P	21/26 80.8%	8/16 50%	0 0%
	P-Value	0.16		

Significant P value ≤0.05.
P1 Val\ Val:Valine\ Valine.
P1 Ile\ Val: Isoleucine\ Valine.
P1 Ile\ Ile: Isoleucine\ Isoleucine.
P: persistent

Discussion:

GSTP1 is a member of the π family and is located on chromosome 11q13, a hot spot for asthma-related genes (Caroll et al, 2005), and is extensively expressed in fetal lung tissue (Beckett et al, 1990).

Aynacioglu et al, 2004 and Mapp et al, 2002 reported the defensive role of GSTP1 in the lung.

Genetic polymorphisms GST P1 is associated with reduced activity of GSTs (Caroll et al, 2005).

This study aimed at identification of GSTP1 polymorphism in children with bronchial asthma using PCR- RFLP technique.

Twenty six cases (59.2%) had Val/Val genotype, 16 cases (36.4%) were Ile/Val and 2 cases (4.5%) were Ile/Ile, which was not significantly different from the control group where 20 cases (66.7%) were Val/Val genotype, 9 cases (30%) were Ile/Val and 1 case (3.3%) was Ile/Ile.

In studying cases versus control results revealed no association between GSTP1 Val/Val, Ile/Val and Ile/Ile and bronchial asthma in children,

P value was statistically insignificant where P value was >0.05.

This study is consistent with Nickel et al, 2005, they concluded that polymorphism within GSTP1 do not represent a major genetic factor in the development of bronchial asthma in German population.

The present study was not consistent with Islam et al, 2009 who found that children who inherit a Val (105) variant allele may be protected from the increased risk of asthma associated with exercise, especially in high ozone communities.

Aynacioglu et al, 2004 they have also reported that the frequency of GSTP1 Val/Val genotype was significantly lower in the group of patients with asthma than in the control individuals.

Moreover Sukuru et al, 2003 results suggested a significant association between GSTP1 Ile/Val polymorphism and susceptibility to asthma and that GSTP1 Val/Val genotype may be protective against the development of the disease.

Caroll et a,l 2005 suggested that GSTP1 Val/Val genotype have an almost 15% higher FEV and FVC than children with GSTP1 Ile/Val or Ile/Ile. genotypes and concluded that GSTP1 is a determinant of lung function.

Anthony et al, (2000) found that the frequency of GSTP1 Val/Val was significantly lower in asthmatics than in control subjects and the presence of this genotype conferred a nine fold lower risk of asthma than did GSTP1 Ile/Ile.

On the other hand, a recent study Cosetta Minelli et al, 2010 findings do not support a substantial role of GST genes in the development of asthma.

Kamada et al, 2007 concluded that GSTP1 gene is a susceptility gene for childhood asthma.

Also Tamer et al, 2004 has found that GSTP1 Val/Val was more prevalent among asthmatic subjects than the control group.

There is evidence suggesting that GST genes, particularly GSTP1 and GSM1, might interact with air pollution and tobacco smoke exposure in exacerbating respiratory symptoms and decreasing lung function in asthmatic individuals (Romieu et al,

2006), (Gilliland et al, 2006).

Peak expiratory flow rate (PEFR) is a useful measure of pulmonary function and is frequently utilized in asthma management. Reduction of PEFR is usually indicative of onset of asthma symptoms. The definition of normal range is always difficult and may vary between regions and is affected by a variety of factors. Factors expected to influence PEF included height, mass, sex, sport intensity and academic grade and smoking (Van Hel den et al, 2001).

We did not found an association between asthma severity and GSTP1 polymorphism, P value was not significant.

Caroll et al, 2005 hypothesised that variation in GST P1 polymorphism may be associated with significant differences in lung function in children and adults.

Also, he suggested that children with GSTP1 Val/Val genotype have an almost 15% higher forced expiratory volume (FEV) and forced vital capacity (FVC) than those with GSTP1 Ile/Val or Ile/Ile. genotypes and concluded that GSTP1 is an important determinant of lung function.

High serum IgE level was detected in our cases with a mean level of (203.45 IU) compared with a lower mean level (107.61 IU) in control cases P value was0.000 which is consistent with Micheal et al, 2005. They reported that total serum IgE levels greater than 100 IU are frequently observed in patients experiencing allergic reactions (Lannier, 2003).

Also, Ottgen and Geha (2001), stated that asthma and predisposition to produce IgE are inherited as linked traits in families and in patients.

Heterogeneity of results as regards GSTP1 could be explained by difference in geographic location for ethnicity, and age of study population. Difference in asthma definition may also have played a role in generating the observed heterogeneity. Endotoxin or other pathogens-associated molecules particularly with gene involved in antioxidant defence such as GST genes may modulate their role in asthma (Von Mutius, 2009) and (London Romieu,2009).

In conclusion, this study demonstrated that GSTP1 polymorphism was not associated with increased risk of bronchial asthma in children.

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الملخص

تعدد أشكال جين الجلوتاثيون- س- ترانسفريزباى والإصابه بحساسية الصدر فى الأطفال

يعتبر الربو الشعبى من الأمراض المزمنة الأكثر شيوعا عند الأطفال وتتراوح نسبة الاصابة به من صفر إلى ٣٠% فى مختلف الشعوب.ويورث الربو الشعبى عن طريق "اضطرابات جينية مركبة".

وقد وجد أن جلوتاثيون- س- ترانسفريز هى مجموعة من الانزيمات اللآيضة التى تتعلق بمنع الاكسدة الناتجة عن الملوثات البيئية والهيدروكربونيات.

ان الاختلافات الوراثية فى كفاءة هده الانزيمات قد تكون عامل وراثى مهم فى امكانية الاصابة بحساسية الصدر.كما ان جلوتاثيون- س- ترانسفريز هى عائلة كبيرة تتضمن الانزيمات الخاصة بالمرحلة الثانية التى تساعد على ازدواج المواد المتفاعلة مع الجلوتاثيون.

وقد تم التعرف على عدة انواع من جلوتاثيون-س- ترانسفريز فى الانسان: الفا- ميو واوميجا.

ان جلوتاثيون- س- ترانسفريز ميووثيتا وباى هم من الجينات التى من وظائفها يتم التعامل مع السموم الداخلية والخارجية وتحويلها الى مواد اقل ضررا ويسهل اخراجها من الجسم، وقد تم التعرف عليها فى عدد كبير من الناس مع الأخذ فى الاعتبار بالاختلافات السلالية.

هدف الدراسه:

الهدف من هذه الدراسة هو الكشف عن التكوين الجينى لجلوتاثيون- س- ترانسفريزباى وعلاقته مع حدوث حساسية الصدر وقد تم التعامل مع ٤٤ حالة من حالات حساسية الصدر ٢٦ من الذكور و١٨ من الإناث وتتراوح أعمارهم من ٥ الى ١٤سنة.

الأدوات:

تم عمل الآتى لجميع الحالات:

أخذ تاريخ مرضى وإجراء فحص إكلينيكى شامل وقياس وظيفة التنفس PEF وفحوص معملية روتينية وأبحاث جينية عن طريق تفاعل البلمرة المتسلسل وتتابع الجينات.

النتائج:

وقد أظهرت النتائج عدم وجود دلالات إحصائية بين تعدد أشكال جين لجلوتاثيون- س- ترانسفريز باى متجانس الازدواج (ليوسين/ ليوسين) أو متباين الازدواج (فالين/ ليوسين) وحساسية الصدر.

الخلاصة:

من هنا نستطيع أن نستنتج من خلال هذه الدراسة عدم وجود زيادة نسبة الإصابة بالربو الشعبى مع وجود تعدد أشكال الجين لجلوتاثيون-س- ترانسفريزباى متماثل الإزدواج أو متباين الأزدواج.

التوصيات:

١. القيام بدراسات مماثلة ولكن على عدد أكبر من الحالات.
٢. دراسة عوامل الاكسدة الناتجة عن الملوثات البيئية والهيدروكربونيات ومدى ودراسة تأثير انزيم الجلوتاثيون- س- ترانسفريزباى على مستوى هذه العوامل فى الرئة.
٣. مقارنة مستيوى انزيم الجلوتاثيون- س- ترانسفريزباى فى الرئة فى مجموعة من الاطفال المصابين بحساسية الصدر ومقارنتهم بمجموعة أخرى من الأطفال الأصحاء.

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Prevalence of Intracranial Hemorrhage In Neonates and Relationship To Obstetric and Neonatal Risk Factors

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Abstract:

Intracranial hemorrhage (ICH) in neonates is an acquired lesion with enormous potential impact on morbidity, mortality, and long term neuro developmental outcome. Despite considerably improved neonatal care and increased survival of preterm infants over recent decades, ICH continues to be a significantly worrisome problem.

Aims:

Aim of this study was to studying the prevalence of ICH in neonates without any neurological signs detected and to determine the different obstetric and neonatal risk factors associated with the development of ICH.

Design:

Case\ control study throughout the period from June 2009 to December 2009.

Subject and Methods:

This case\ control study was conducted on 120 neonates who were admitted to Neonatal Intensive Care Unit (NICU) of Obstetrics and Gynecology Hospital, Ain Shames University. Detailed history taking: maternal, obstetric and delivery circumstance laying stress on maternal and obstetric risk factors of ICH. Assessment of the general condition using Apgar score at 1, 5 and 10 minutes. Assessment of gestational age (GA) using Ballard score. Assessment of birth weight. Thorough clinical examination laying stress on neurological examination according to Sarnat and Sarnat. Imaging studies using cranial ultrasound (CUS) at 1, 3, 7 days of life and on discharge. Brain computed tomography (CT) scan in neonates suffered from instrumental delivery

Results:

Out of 120 neonates 39 neonates developed ICH (32.5%). 42.9% male, 21.1% female. Intraventricular hemorrhage (IVH) was the commonest one 24.2%, grad one IVH represent 13.4%. The prevalence of asymptomatic cases with ICH was 66.7%. There are certain maternal risk factors that are associated with increase risk of ICH, in which prolonged delivery

assisted by forceps and vacuum extraction was 43.58%, 15.38% was premature rupture of membrane (PROM), 12.83% was malpresentation. The neonatal risk factors that are associated with increase risk of ICH where prematurity representing 67.4%, 76.9% pneumothorax, 77.3% trauma. The sensitivity of CUS for detection of ICH was 84.6%, the specificity of CUS was higher representing 97.5%. 41.03% of cases with ICH were appeared in the 1st day of life, 76.9% in 3rd day by CUS.

Conclusion:

The prevalence of asymptomatic cases with ICH was 66.7%. There are certain maternal and neonatal risk factors that are associated with increase risk of ICH. CUS can be considered as a specific and sensitive indicator for occurrence of ICH.

Introduction:

Intracranial hemorrhage (ICH) in neonates is an acquired lesion with enormous potential impact on morbidity, mortality, and long-term neurodevelopmental outcome (Bassam, 2009). ICH is an uncommon but important cause of morbidity and mortality in term neonates (Ou-Yang et al., 2010).

Bleeding within the skull can occur external to the brain into the epidural, subdural or subarachnoid space, into the parenchyma of the cerebrum or cerebellum or, into the ventricles from the subependymal germinal matrix (GM) or choroids plexus (Volpe, 2001).

Of all types of ICH, germinal matrix-intraventricular hemorrhage (GM-IVH) is the most common and distinctive pathology (Volpe, 2008). Periventricular intraventricular hemorrhage (PIVH) is a major cause of neurological disabilities in preterm newborns (Lee et al., 2010).

ICH following vaginal birth in neonates appear to be common, with a prevalence of 26% (Loony et al., 2007). The prevalence of ICH was 24%, subdural hemorrhage (SDH) was the most prevalent type of ICH in term infant (Zakhary et al., 2009).

Diagnosis typically depends on clinical suspicion, when an infant presents with typical neuralgic signs such as, seizures, irritability, or depressed level of consciousness and or with focal neuralgic deficits referable either to the cerebrum or brain stem (Soul, 2008). The associated clinical signs of IVH are typically non specific or absent, therefore it is recommended that premature infants < 34 week GA should be evaluated with routine real time CUS through the anterior fontanel to screen for IVH within the first 3-5 days of age. CUS is the preferred imaging technique for screening because it is non invasive, portable reproducible, sensitive and specific for detection of IVH (Kliegman et al., 2008) (Guillerman, 2010) (Khan et al., 2010) (Miranda., 2010)

Aims:

1. Studying the prevalence of ICH in neonates without any neurological signs detected
2. Assessment of different obstetric and neonatal risk factors associated with the development of ICH.

Patients And Methods:

This case\ control study was conducted on 120 neonates who were admitted to Neonatal Intensive Care Unit (NICU) of Obstetrics and Gynecology Hospital, Ain Shames University throughout the period from June 2009 to December 2009. The studied neonates were divided into two groups:

1. The patient group.
2. The control group.

Inclusion Criteria:

1. Full term and preterm neonates
2. Neonates admitted to neonatal ICU with assisted vaginal delivery (forceps or vacuum extraction) and prolonged duration of labor, premature rupture of membrane, birth asphyxia.

Exclusion Criteria:

1. Extreme low birth weight newborns (<1000 grams of birth weight).
2. Congenital malformation of the brain
3. Neonatal sepsis such as meningitis, encephalitis

Methods:

All neonates were subjected to:

1. Detailed history taking: maternal, obstetric and delivery circumstance laying stress on maternal and obstetric risk factors of ICH e.g. hypertension. diabetes mellitus, pulmonary disease malpresentation, multiple pregnancy, Polyhydraminous, placental abnormalities, premature rupture of membranes (PROM), prolonged labor, assisted vaginal delivery (forceps or vacuum extraction)
   a. Assessment of the general condition using Apgar score at 1, 5 and 10 minutes (Apgar, 1953).
   b. Assessment of gestational age using Ballard score (Ballard et al., 1991).
   c. Anthropometric measurement (birth weight, head circumference)
2. Thorough clinical examination laying stress on neurological examination according to (Sarnat and Sarnat, 1976)
   a. Assessment of the need of the ventilator support.
3. Investigations:
   a. Laboratory:
      - Complete blood count (CBC): To exclude sepsis (using Coulter- counter model T 660- Coultronics, France).
      - C- reactive protein: Latex agglutination test, Avitex- CRP, Omega diagnostic limited.
      - Principle: An immunologic reaction between CRP as an ntigen and the corresponding antibody coated on the surface of biologically inert Latex particles (Fischet., 1967).
      - Arterial blood gases (ABG) analysis (blood gas analyzer Mod. 995, Hb Trust Medical Company).
   b. Cranial ultrasound: Trans-cranial ultrasound was used via anterior fontanel using GE LOG IQ3 probe, using 8 MHz probe, 8C (convex), 8L (linear).

   Ultrasound was performed for all neonates on 1, 3, 7 days & on discharge using A general electric (GA) sector scanner with rotating trasducer. the transducer is placed on the anterior fontanell to obtain signals from the anterior cerebral artery (ACA) and between the eye socket & the ear, just above the zygoma to obtain signals from the meddle cerebral artery (MCA). The back -scatter signal are subjected to real-time spectral analysis & then displayed visually as a sonogram on a monitor, & as an auditory signal. This method allows the optimal signals to be obtained as maintained during the recording when the image is stored, the resistive index (RI) is calculated by analyzing the maximum frequency envelop from at least five consecutive cardiac cycles to give peak or systolic frequency (S)& through or diastolic frequency (D). This had to be repeated two or three times to ensure reproducibility. The RI is derived from the equation:

$$RI = \frac{(S- D)}{S}$$

The findings were classified as abnormal when: Areas of echo densities were seen in the periventricular or subcortical white matter, ventricular size showing mild dilatation (0.5- 1.0 cm), moderate (1.0- 1.5) and sever dilatation (> 1.5

cm). RI obtained is above 0.700

PIVH was graded into 4 grades according to the (Papile et al., 1978) grading:

- Grade I: Isolated GMH (no PIVH)
- Grade II: PIVH without Ventricular dilation
- Grade III: PIVH with Ventricular dilation
- Grade IV: PIVH with parenchymal hemorrhage

C- Computed Tomography (CT) Scans of the brain: An additional imaging study to neonates with traumatic delivery shows abnormality in the form of hyperdense areas

Statistical Analysis:

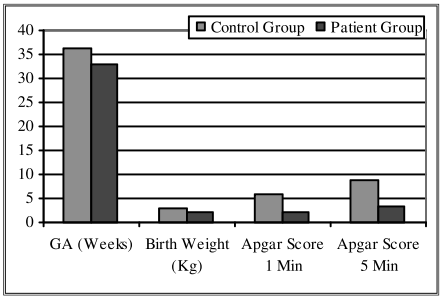
The data were coded, entered and processed on computer using SPSS (version 15). The level $P < 0.05$ was considered the cut-off value for significance.

1. Chi-Square test (X^2) was used to test the association variables for categorical data.
2. Fisher exact test was performed in table containing value less than 5.
3. Student's T-Test was used to assess the statistical significance of the difference Between two population means in a study involving independent samples.
4. Multivariate Logistic regression analysis is useful for situations in which you want to be able to predict the presence or absence of a characteristic or outcome based on values of a set of predictor variables
5. The correlation coefficient method was used to relate different parameter
6. Probability (P) value, P value was used as determinant as significance (If P> 0.05= Insignificant If P< 0.05= significant If P< 0.01= highly significant).
7. Graphic presentation of the results was also done.

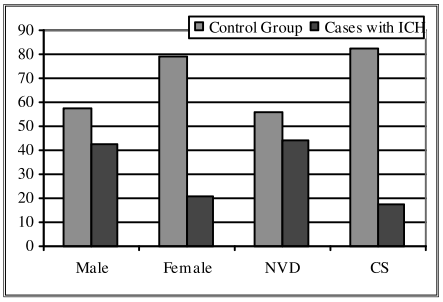
Results:

This case\ control study was conducted on 120 newborns, were admitted to the NICU of Gynecology and obstetric Hospital Ain Shams University Hospital during the study period from June 2009 to December 2009. Thirty nine of them (32.5%) were diagnosed as patient group in which 27 cases (42.9%) male, 12 cases (21.1%) female, 30 cases (44.1%) delivered vaginally, 9 cases (17.3%) cesarean section, their mean gestational age was 32.82 weeks, and their mean birth weight was 1.95 Kg, their mean Apgar score at 1 minutes was 2.00 and at 5 minutes was 3.38. Eighty one were normal or control groups in which 36 of them (57.1%) male, 45 of them (78.9%) female, 38 of them (55.9%) delivered vaginally, 43 (82.7%) cesarean section their mean gestational age was 36.33 weeks, and their mean birth weight was 3.03 Kg, their mean Apgar score at 1 minutes was 5.75 and at 5 minutes was 8.56 (figure 1 and 2).

Figure (1): GA, birth weight, Apgar score



Figure(2): Sex, MOD among patient and control group



Percentage of cases with ICH was 32.5% (39cases) in which IVH was the commonest representing 24.2% (29 cases), SAH was 2.5%

(3cases), SDH was 2.5% (3 cases), IPH was 3.3% (4 cases). Grad one IVH was the commonest representing 13.4% (16 cases), grade II was 4.2% (5 cases), grade III 3.3% (4 cases), grade IV 3.3% (figure 2)

Figure(3): Percentage of cases with ICH

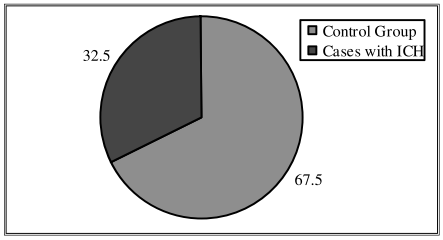
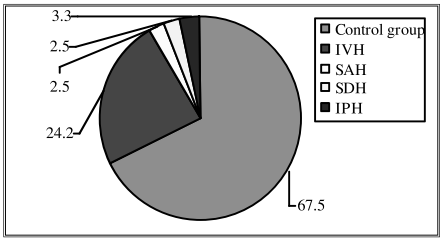


Figure (4): Frequency distribution of types of ICH



There are certain maternal factors that are associated with increase risk of ICH, including, prolonged and instrumental delivery by forceps and ventuse extraction were the commonest representing 43.58%, 15.30% was PROM, 12.83% was male presentation (table 1)

Table (1) Maternal risk factors among mothers of cases with ICH

Maternal Risk Factors	no	%
Toxemia Of Pregnancy	3	7.69%
Diabetic Vasculopathy	2	5.12%
Pulmonary Disease	1	2.57%
PROM	6	15.38%
Prolonged and vacuum - forcipes delivery	17	43.58%
Mal Presentation	5	12.83%
Multiple Pregnancy	3	7.69%
Polyhydraminous	1	2.57%
Placental Abnormality	1	2.57%

PROM= premature rupture of membrane

There are certain neonatal factors that are associated with increase risk of ICH, including, pneumothorax, prematurity, trauma, nonvertex presentation, ventlatory use, lower blood pressure, increased CO_2 (table 2)

Table (2A) :Neonatal risk factors among cases with ICH and control group

Neonatal Risk Factors	Control Group		Cases With ICH		X^2	P	Sig.
	no	%	no	%			
Prematurity	14	32.6	29	67.4	37.30	<0.0001	HS
Pneumothorax	6	23.1	20	76.9	29.86	<0.0001	HS
Trauma	5	22.7	17	77.3	24.62	<0.0001	HS
Non Vertex Presentation	18	40.0	27	60.0	24.83	<0.0001	HS
Ventilatory Use	22	27.2	22	56.4	9.70	0.002	HS

Table (2B): Neonatal risk factors among cases with ICH and control group

Neonatal Risk Factors	Control Group		Cases With ICH		T	p	Sig.
	Mean	± Sd	Mean	± Sd			
SBP	67.02	± 3.08	51.23	± 1.47	21.72	<0.0001	HS
DBP	43.94	± 3.30	33.72	± 2.06	12.32	<0.0001	HS
Increase CO_2	37.21	± 7.21	61.85	± 4.16	13.88	<0.0001	HS

SBP= Systolic blood pressur
DBP= Diastolic blood pressure, CO_2= Carbon dioxide

In the current study it was found that the neurological signs were detected in 33.3% of cases with ICH while 66.7% were asymptomatic (table 3).

Table (3): Neurological signs among cases with ICH and control group

Neurological Signs	Control Group		Cases With ICH		X^2	P	Sig.
	no	%	no	%			
Negative	68	83.95	26	66.7	4.63	0.03	S
Positive	13	16.05	13	33.3			

Cases with ICH shows significantly differences in all blood gases parameters in the form of acidosis, hypoxia, hypercarbia, low bicarbonate in comparison to control group (table 4)

Table (4): Blood gases parameters among cases with ICH and Control group

Blood Gases parameters	Control Group		Cases With ICH		t	p	Sig.
	Mean	± Sd	Mean	± Sd			
PH	7.32	±0.08	7.12	±0.18	4.63	<0.0001	HS
PCO_2 (Mmhg)	37.21	±7.21	61.85	±4.16	13.88	<0.0001	HS
PO_2 (Mmhg)	58.26	±4.75	33.40	±3.36	20.05	<0.0001	HS
HCO_3(mEq/L)	22.21	±1.80	16.71	±0.90	12.81	<0.0001	HS
Base Deficit	5.69	±1.26	16.14	±3.78	12.75	<0.0001	HS

On studying CBC their was highly significant lower Hb, RBCs, HCT, and platelets in cases with ICH but their was no statistical significant differences as regard WBCs in comparison to control group (table 5)

Table (5): Complete blood counts among cases ICH and control group

CBC	Control Group		Cases With ICH		t	p	Sig.
	Mean	± Sd	Mean	± Sd			
Wbcs × 103/Cmm	14.58	± 5.05	13.66	± 4.10	0.99	0.32	NS
Rbcs × 106/Cmm	4.58	±0.73	3.80	±0.58	5.85	<0.0001	HS
Hb (g/dl)	15.56	± 2.98	11.92	± 2.01	6.90	<0.0001	HS
Hct%	42.07	± 8.61	34.40	± 4.33	5.25	<0.0001	HS
Platelet × 103/Cmm	249.70	± 64.40	145.21	± 89.57	7.30	<0.0001	HS

On studying the correlation between birth weight, GA and ICH it was found that there was a significant negative correlation (table 6).

Table (6): Correlation between birth weight, gestational age and ICH.

	r	p	Sig.
Birth Weight	-0.672	0.01	S
Gestational Age	-0.730	<0.001	S

r= Correlation

As regard the correlation between cases with ICH and blood gases parameters it was found that there was an significant negative correlation in Ph, PO_2, HCO_3 and positive correlation with PCO_2 (table 7)

Table (7): Correlation between cases with ICH and blood gases parameters

	r	p	Sig.
Ph	-0.601	.001	S
PCO_2	+0.721	<0.001	S
PO_2	-0.609	0.002	S
HCO_3	-0.593	0.01	S
Base Excess	+0.579	0.02	S

Multivariate logistic regression analysis was performed to identify the predictor factors, it demonstrated that GA and obstetric risk factors were the most predictor factors for ICH (P <0.05). The risk of ICH was nearly 6 folds in group with obstetric risk factors compared to those without obstetric risk factors, hazard ratio was 6.11, 95%CI (2.12 to 17.6) P=0.001 and 5folds for GA less than 34 weeks than those > 34 weeks GA, hazard ratio was 5.06, 95% CI (1.82 to 14.08) (P=0.002). (table 8)

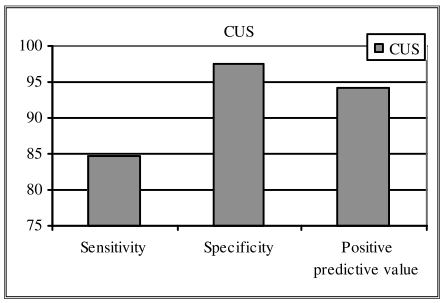
Table (8): Risk factors for ICH (using multivariate logistic regression analysis).

		p	HR	95.0% CI
Sex	Female [1]			
	Male	0.18	1.89	0.75 - 4.75
Mode Of Delivery	Cs [1]			
	NVD	0.09	2.34	0.88 - 6.19
Obstetric Risk Factors	-Ve[1]			
	+Ve	0.001	6.11	2.12 - 17.60
Gestational Age	>34			
	<34	0.002	5.06	1.82 - 14.08

[1] HR= Hazard Ratio, CI=Confidence Interval

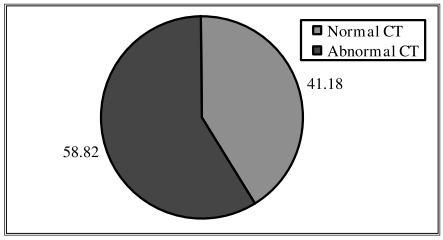
On Studying CUS finding it was found that 41.03% of cases of ICH were appeared in the first day of life, 76.9% appeared on third day of life, 84.6% appeared at one weak, on discharge it was found that 80.8% of cases of ICH were resolved on CUS, 19.2% of cases showed changes of squeals. The sensitivity of CUS for detection of ICH was 84.6%, the specificity of CUS was higher representing 97.5% and positive predictive value of CUS for detection of ICH was 94.3% (figure 5)

Figure (5)::Sensitivity, Specificity and Positive predictive value of CUS for detection of ICH



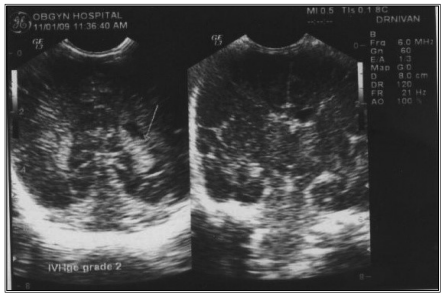
On studying CT finding on neonates that exposed to instrumental delivery by forceps and vacuum extraction, 58.8% from them suffered from ICH (SAH, SDH, IPH) (figure 6)

Figure (6): Frequency of abnormal CT findings among neonate with traumatic delivery

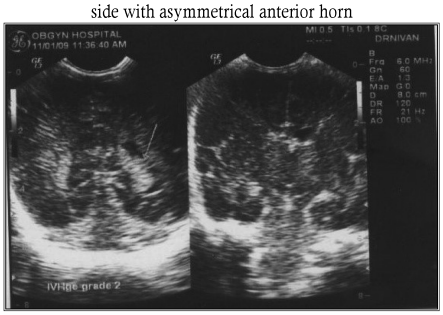


Mortality rate was 33.3% in cases with ICH in comparison to control group was 14.8%

Figure(7:) CUS showing grade I IVH, Asymmetrical anterior horn, bulky Choroids plexus on left side with Bilateral IVH Grade I



Figure(8): CUS showing gradeII IVH, unilateral IVH on left side with asymmetrical anterior horn



Figure(9): CUS showing grade III IVH, Dilatation of lateral ventricle, 3rd and 4th Ventricle bilaterally

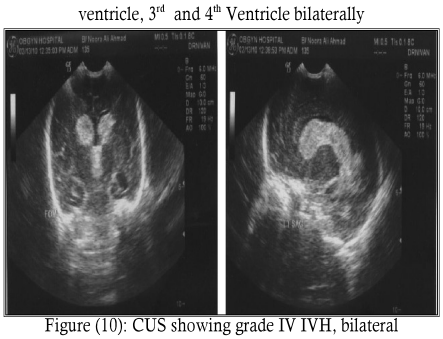


Figure (10): CUS showing grade IV IVH, bilateral intracranial foci at the occipital region, ventricular dilatation of both lateral Ventricles especially on left side

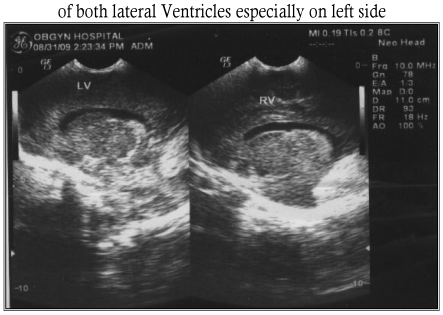


Figure (11): CT showing SAH, IVH, hyperdense minimal lateral rim of the subarachnoid space (arrow).

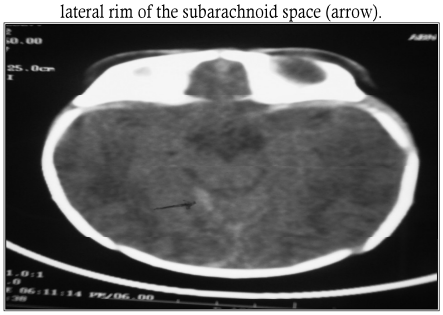


Figure (12): CT showing SDH, hyperdense concavo-convex lesion in the right fronto-parietal region

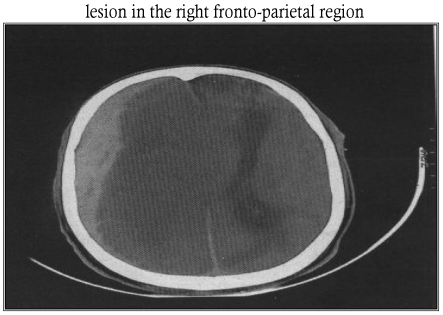
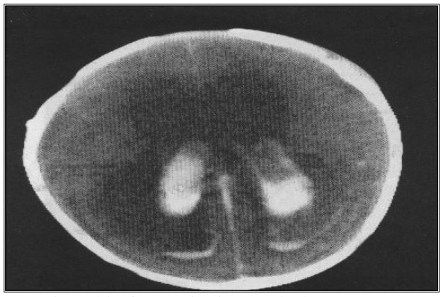


Figure (13): CT showing hyper dense area in ventricles with ventricular dilatation



Figure(14): CT showing intracerebral hemorrhage, Hyperdense area in the left fronto-parietal region hemisphere

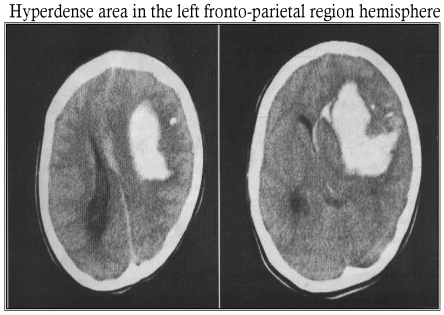
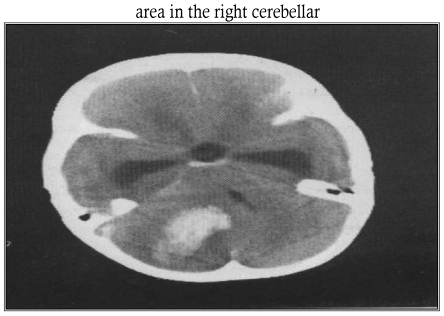


Figure (15): CT showing cerebellar hemorrhage, hyperdense area in the right cerebellar



Discussion:

This case- control study was conducted on 120 newborns, were admitted to the NICU of Gynecology and Obstetric Hospital Ain Shams University during the study period from June 2009 to December 2009. There were 42.9% males, 21.1% females had ICH, in which their mean GA was 32.82 weeks and their mean birth weight was 1.95 Kg.

As regard the GA we found that there was highly significant lower GA among cases with ICH (P<0.0001) this is in agreement with (Dolfin et al., 1994) who was reported that lower GA was associated with greater risk of high IVH occurrence. The same relation was shown by (Khodapanahandeh et al., 2008) (Roze et al., 2008) (Lim et al., 2009) (Lee et al., 2010) (Miranda., 2010).

In this study it was found that there was a significant lower birth weight among cases with ICH in comparison to the control group (P=0.01). This finding was in agreement with the study done by (Dykes et al., 2000) who was found that, birth weight less than or equal to 1,200 gm, were associated with PIVH. The same relation was shown by (Patra et al., 2006) (Khodapanahandeh et al., 2008) (Baumert et al., 2008) (Brouwer et al., 2010) (Mohamed et al 2010) (Lee et al., 2010) (Miranda., 2010).

As regard the Apgar score it was found that there was a highly significant lower Apgar score at one minute and 5 minutes among cases with ICH (P<0.0001). This finding was in agreement with (Baumert et al., 2008) who was reported that lower Apgar score was associated with greater risk of high IVH occurrence. The same relation shown by (Khodapanahandeh et al., 2008) (Brouwer et al., 2010) (Lee et al., 2010)

On studying the gender relation to ICH it was found that male gender showed significantly higher

rate of ICH than female (P=0.01) in which 52.5% of total studied newborns were males, 42.9% from them had ICH. While 47.5% were females, 21.1% from them had ICH. This finding was in agreement with (Dykes et al., 2000) who was found that the IVH was significantly higher in male compared with female neonates. The same relation shown by (Tioseco, , et al., 2006) (Baumert et al., 2008) (Cuestas et al., 2009) (Mohamed et al 2010) (Ou-Yang et al., 2010).

It was found that among cases delivered vaginally, 44.1% developed ICH while only 17.3% of cases delivered by CS had ICH. This finding was in agreement with Towner., 1999 who was reported that vaginal delivery with forceps assistance, vetoes extraction were associated with an increased risk for ICH. The same relation shown by (Wen et al., 2001) (loony et al 2007) (Kicklighater et al., 2007) (Fuijkschot et al., 2008) (Baud, 2008) (Villarejo et al., 2009) (Lee 2010).

In the present study out of 120 neonates in NICU, 39 neonates developed ICH (32.5%). Volpe, 2001 stated that the incidence of ICH varies from 2% to > 30% in newborns depending on the GA at birth and the type of ICH. This was in agreement with the study done by (Loony et al., 2007) who reported 26%. (Zakhary et al., 2009) reported 24%

On studying the different types of ICH it was found that 24.2% had IVH 2.5% had SAH, 2.5% had SDH, 3.3% had IPH. In which IVH was the commonest. In agreement with our finding, study don by (Andrerson et al., 1994) who was found that IVH was diagnosed in 21% of neonates born at 32 weeks gestation or less. (Dolfin et al., 1994) was found that the incidence of PIVH was 45% in infants ≤ 29 weeks gestation, whereas 19% in infants > 29 weeks gestation. (Volpe, 2001) reported that IVH was 15%- 20 (Hamrick et al., 2004) reported that IVH was 15%- 25%. (Tioseco et al., 2006) reported that IVH was 12.2% males versus 7.2% females. (Mc Crea et al., 2008) found that IVH occurs in 20 to 25% of VLBW preterm neonates. (Cuestas et al., 2009) found that the rate of over all IVH was 26.8% males versus 9.7% females (Bassan, 2009) stated that of all types of ICH, GM -IVH is the most common. (Guzman Cabanas et al., 2009) found that sever IVH was diagnosed in 8.5%. (Lee et al., 2010) was found that PV-IVH was 27.8%.

On studying frequency of different grades of IVH it was found that 13.4% had grade I, 4.2% had grade II, 3.3% had grade III, 3.3 had grade IV. This was in agreement with (Kadri et al., 2006) who was found that The incidence of PIVH among preterm neonates was 44.68%. The majorities were mostly grade I and II. In contrast to (Kliegman et al., 2008) who stated that grade I= 35% of IVH cases, grade II= 40% of IVH cases. (Lee et al., 2010) was found that 79.7% with grad I IVH, 6.9% grad II, 4.8% grad III and 8.6% grad IV

There are certain maternal factors that are associated with increase risk of ICH, including, prolonged and instrumental delivery by forceps and ventuse extraction were the commonest representing 43.58%, 15.30% was PROM, 12.83% was male presentation, other maternal risk factors were less common. These findings were in agreement with (Loony, 2007) who was reported that there were several factors had been reported to increase the risk of ICH newborns, these factors includes assisted vaginal delivery (forceps or vacuum extraction), maternal parity, fetal weight, PROM and prolonged duration of labor. The same relation shown by (Roze et al., 2008) (Villarejo et al., 2009) (Lee et al., 2010) (Schulze et al., 2010)

There are certain neonatal factors that are associated with increase risk of ICH, including, pneumothorax, prematurity, trauma, nonvertex presentation, ventlatory use lower blood pressure, increased CO_2. These findings were in agreement

with (Dykes et al., 2000) who stated that hypercarbia, hypotension, associated with PIVH. (Khodapanahandeh et al., 2008) reported that the following factors were associated with greater risk of high IVH occurrence: lower GA, lower birth weight, mechanical ventilation, HMD, symptomatic hypotention, hypercapnia and lower Apgar score at 5 minutes. The same relation shown by (Roze et al., 2008) (Gupta et al., 2009) (Perlman, 2009) (Kaiser, 2009) (Lee et al., 2010) (Crowther et al., 2010).

In the current study it was found that the neurological signs were detected in 33.3% of cases with ICH while 66.7% were asymptomatic. This finding were in agreement with (Loony et al., 2007) who found that there was high prevalence of ICH in asymptomatic newborns, ICH causes more subtle injury to the developing brain. (Rooks et al., 2008) share the same opinion.

As regard CBC it was found that there was a highly significant lower RBCs, Hb, HCT and platelet among cases with ICH in comparison to control group (P<0.0001). In the present study it was found that the mean of platelet was 145.21% ±89.57. This was in agreement with (Jhawer et al., 2003) who was found that thrombocytopenia is the most common condition associated with ICH, (Brouwer et al., 2010) was found that 22% of infants had a platelet count less than 150×10^9 L.

On studying the correlation between birth weight, GA and ICH it was found that there was a significant negative correlation. These findings were in agreement with (Kadri et al., 2006) who stated that the incidence of IVH among preterm neonates was inversely related to the weight and the age of the newborns. (Braumer et al., 2010) was found that birth weight< 10^{th} percentile were more at risk for ICH (Miranda., 2010) was found that the incidence of IVH increases as the GA decreases, higher-grade hemorrhages occur more frequently in low -birth weight neonates.

As regard the correlation between cases with ICH and blood gases parameters it was found that there was an significant negative correlation in PH, PO_2, HCO_3 and positive correlation with PCO_2.These finding were in agreement with study done by (Dykes et al., 2000) who was found that hypoxia, hypercarbia and acidosis were associated with increase risk of PIVH. The same relation was reported by (Bassan et al., 2005) (Kachi et al., 2006) (Roze et al., 2008) (Kaiser, 2009).

Multivariate logistic regression analysis was performed to identify the predictor factors, it demonstrated that GA and obstetric risk factors were the most predictor factors for ICH (P <0.05). The risk of ICH was nearly 6 folds in group with obstetric risk factors compared to those without obstetric risk factors, hazard ratio was 6.11, 95%CI (2.12 to 17.6) P=0.001 and 5folds for GA less than 34 weeks than those > 34 weeks GA, hazard ratio was 5.06, 95%CI (1.82 to 14.08) (P=0.002). These findings were in agreement with study done by (Roze et al., 2008) who was found that GA and maternal intrauterine infection were as predictors with hazard ratio 12.2, 95%CI: 1.2- 127.0 (P=0.04). The same relation shown by (Khodapanahandeh et al., 2008).

On Studying CUS finding it was found that 41.03% of cases of ICH were appeared in the first day of life, 76.9% appeared on third day of life, these findings were in agreement with (Hellstrom-Westas et al., 2001) who was stated that postnatally, most hemorrhage occur when the neonate is younger than 72 hours, with 50% hemorrhage occurring on the first day of life. The extent of hemorrhage is greatest when the neonate is aged approximately 5 days. Similar results reported by (Kadri et al., 2006) (Volpe, 2008) (Khodapanahandeh et al., 2008).

The sensitivity of CUS for detection of ICH was 84.6%, the specificity of CUS was higher

representing 97.5% and positive predictive value of CUS for detection of ICH was 94.3%, this finding were in agreement with (Khan et al., 2010) who stated that CUS examination provides a relatively sensitive and highly specific means of detecting IVH. CUS is not reliable in the detection of non matrix related hemorrhage. CT was found to be more sensitive in detecting extra axial hemorrhage. CT was also better in detection non matrix related IPH.

In the present study on discharge it was found that 80.8% of cases of ICH were resolved on CUS, 19.2% of cases showed changes of squeals, this finding was in agreement with (Roze et al., 2008) who was found that periventircular hemorrhagic infarction (PVHI) was seen in 10 to 15% of preterm infants with GMH. (Lee et al., 2010) was found that PV-IVH is one of the major causes of the development of neurological impairment and the incidence ranges from 15% to 40%.

On studying CT finding on neonates that exposed to instrumental delivery by forceps and vacuum extraction, 58.8% from them suffered from ICH (SAH, SDH, IPH).This finding were in agreement with (Loony et al., 2007) who reported that assisted vaginal delivery (forceps or vacuum extraction) increase the risk of ICH which includes SAH, SDH, IPH, GMH. The same relation shown by (Kicklighater et al., 2007) (Fuijkschot et al., 2008) (Baud, 2008) (Villarejo et al., 2009) (Lee, 2010).

In the present study it was found that the mortality rate of total neonates was 20.8%. 33.3% among cases with ICH, in which the higher mortality rate (41.4%) was found in cases with IVH and 50% in IPH (P=0.001). (Gomella, 2004) was reported that the mortality rate in cases with mild to moderate PIVH was 5-10%, 20% with sever PIVH, 50% with severe PIVH and parenchymal involvement. (Synnes et al., 2006) reported 27-50%.(Yilmaz et al., 2009) reported 33%. (Vassilyadi et al., 2009) reported 20%, (Brouwer et al., 2010) reported 24.5%. (Lee, 2010) reported 35.6%.

In the current study blood gases parameters showed statistical significant difference between patient group and control group (P<0.0001) especially hypoxia, hypercarbia, acidosis among cases with ICH. This finding was in agreement with (Dykes et al., 2000) who was reported that hypoxia, hypercarbia were associated with PIVH. The same relation showen by (Bassan, 2005) (Kadri et al., 2006) (Khodapanahandeh et al., 2008) (Kaiser et al., 2005, 2006 and 2009). (Roze et al., 2008) reported that the perinatal and neonatal risk factors including umbilical cord PH < 7.1 increase the risk of ICH, the same relation shown by (Lee et al., 2010)

Conclution:

The prevalence of asymptomatic cases with ICH was 66.7%. There are certain maternal and neonatal risk factors that are associated with increase risk of ICH. CUS can be considered as a specific and sensitive indicator for occurrence of ICH.

Recommendations:

Good perinatal care, avoid instrumental delivery. Routine screening using CUS are recommended for all infants born at 34 weeks' gestation or earlier and for all VLBW infants (<1500 grams of birth weight).

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