INTRODUCTION

Matrix metalloproteinase (MMP), are super family of 23 human zinc- dependent endopeptidase involved in tissue remodeling, cell migration, angiogenesis, activation of growth factors, and regulation of inflammation and ulceration ***(Khalid and Javaid,2016)***

MMP_s are expressed by macrophages, neutrophils, fibroblastic cells, vascular smooth muscle cells, migrating keratinocytes, and osteoclasts ***(Khalid and Javaid,2016).***

MMP-2 and MMP-9 collectively known as the gelatinases, are particulary important in the pathogenesis of inflammatory, infectious, and neoplastic diseases in many organs including the lung and intestine ***(Kim et al., 2016).***

MMP-2 and -9 plasma activities and tissue inhibitor of metalloproteinase-1 (TIMP-1) and -2 plasma concentrations in preterm and term neonates were dependent on the gestational and postnatal age. Matrix-metalloproteinases (MMP), play crucial role in numerous pathological processes, because cellular changes may be reflected in body fluids, measurements of MMP in blood have been recommended as non invasive tools in diagnosis and monitoring of diseases ***(Klaus et al., 2008).***

Critically ill neonates, when mother has a high risk pregnancy, there is a possibility that her baby could be born prematurely as < 37 weeks, and would need to be placed in the neonatal intensive care unit (NICU). The care of a critically ill neonate in the emergency department will provide guidelines

for the initial stabilization of these infants as well as identify differential diagnosis that should be considered. As with any critically ill patient, achieving physiologic stability of the neonate is the first priority. However, in addition to maintaining the airway, breathing, and circulation, thermo neutrality must be achieved for a successful outcome ***(Kim et al., 2008).***

Bronchopulmonary dysplasia (BPD), is a common lung problem among preterm infants, especially those less than 1.000g at birth. The exact mechanism for this disease more or less are actually so, many things are cleared now, but extreme prematurity, severe respiratory distress syndrome (RDS), infection before and after birth, and the prolonged use of oxygen and / or a ventilator needed to treat a lung disease all play a major role in the development of BPD **(Schmidt, *2015).***

Bronchopulmonary dysplasia (BPD) is defined as the need for supplemental oxygen for at least 28 days after birth in infants were born < 32 weeks. For infants were born at ≥ 32 weeks, BPD is defined as a supplemental O_2 requirement for first 28 days with severity level based on oxygen requirement at 56 days ***(Bancalari and Claure, 2016).***

at 36 weeks' postmenstrual age (PMA) (PMA= Gestational age + Chronological age), **mild BPD** is defined as no supplemental oxygen requirement, **moderate BPD** is defined as requirement of supplemental O_2 <30%, **severe BPD** is defined as requirement of ≥ 30% O_2 and / or continuous positive airway pressure (CPAP) or ventilator support ***(Wright et al., 2016).***

Inflammation is a major contributor to the pathogenesis of BPD. Often initiated by a pulmonary fetal inflammatory response, lung inflammation is exacerbated by mechanical ventilation and exposure to supplemental oxygen. In response to these initiators of injury, a complex interaction occurs between proteins that attract inflammatory cells (i.e. chemokines), proteins that facilitate the trans endothelial migration of inflammatory cells from blood vessels (i.e. adhesion molecules), proteins that promote tissue damage (i.e. pro-inflammatory cytokines and proteases) and proteins that modulate the process (e.g. anti-inflammatory cytokines, binding proteins and receptor antagonists) ***(Bose et al., 2008).***

In addition, during recovery from inflammatory injury, growth factors and other substances that control normal lung growth and mediate repair influence subsequent lung structure **(*Bose et al., 2008).***

Necrotizing enterocolitis (NEC), is primarily a disease process of the gastrointestinal tract of premature neonates that results in inflammation and bacterial invasion of the bowel wall. Despite advances in the care of premature neonates, NEC remains one of the leading causes of morbidity and mortality in the population ***(Thampson and Bizzarro, 2008).***

It occurs in 1-5% of all neonates' intensive care admissions and 5-10% of all very low birth weight (1500 g) infants. Although research has presented an interesting array of potential contributing factors. The precise etiology of this multifactorial disease remains elusive ***(Thampson and Bizzarro, 2008).***

Neonatal sepsis, is a serious medical condition that is characterized by a whole body inflammatory state called systemic inflammatory response syndrome (SIRS) in the presence of a know or suspected infection. The body may develop this inflammatory response to microbes in the blood, urine, skin or other tissues ***(Dellinger et al., 2007).***

Prematurity is single most significant factor correlated with sepsis. Neonatal sepsis can be classified into two relatively distinct syndromes based on the age of presentation into early-onset sepsis and late-onset sepsis ***(Ray et al., 2006).***

Importance of the study:

Matrix-metalloproteinases (MMPs), play crucial role in numerous pathological processes. The MMPs are implicated in the pathogenesis of some diseases during neonatal period which are recognized to be causes of severe long term neurodevelomental deficits in children.

AIM OF THE STUDY

This study, aimed at:

1. Evaluation of the role of matrix metalloproteinase-9 during the pathogenesis of bronchopulmonary dysplasia, necrotizing enterocolitis and neonatal sepsis.
2. Studying the relation between matrix metalloproteinase-9, diseases severity, and short term outcome.

CRITICALLY ILL PRETERM NEONATES

Preterm birth: is defined as the birth of a baby before the developing organs are mature enough to allow normal postnatal survival ***(Martin et al., 2013).***

Critically Ill Preterm: they are three groups of high risk neonates always present with difficult treatment decisions for their parents and pediatricians:

a) Infants born between 22-25 gestational weeks, considered at the threshold of viability.

b) Infants born with major and/or multiple congenital abnormalities.

c) Infants severely damaged by complications of pregnancy, delivery, or the early neonatal period ***(Ho and Goh, 2013).***

Classification as one of the following based on gestational age:

- Extremely preterm (< 28weeks)
- Very preterm (28 to <32weeks)
- Moderate to late preterm (32 to < 37weeks) ***(Women's and Childern's Health, 2014).***

Approximately 12.5% of births are preterm (occurring before 37 weeks of gestation). Preterm infants with "very low " birth weight are those who weigh 1500 g or less; those with " extremely low " birth weight weighs 1000 g or less.Although they account for only 1.5% and 0.7% of live births, respectively

(Eichenwald and Stark, 2008). Due to the immaturity of the organs at the time of birth, preterm infants exhibit an increased risk of developing a number of postnatal complications including renal insufficiency and in severe cases renal failure, etc…., .

The critically ill neonates include:

- **Respiratory distress syndrome (RDS):** Rspiratory distress syndrome develops because of impaired surfactant synthesis and secreation leading to atelectasis, ventilation-perfusion disfunction (V/ Q) inequality, and hypoventilation with resultant hypoxemia and hypercarbia. Blood gases show respiratory and metabolic acidosis that cause pulmonary vasoconstriction, resulting in impaired endothelial and epithelial integrity with leakage of proteinaceous exudate and formation of hyaline membranes (hence the name) (***Sun et al., 2015).***
- **Neonatal Sepsis (NS):** Sepsis is a systemic inflammatory response, remains one of the leading causes of morbidity and mortality both among term and preterm infants ***(Gonzalez et al., 2013).*** Although advances in neonatal care have improved survival and reduced complications in preterm infants, sepsis still contributes significantly to mortalitiy and morbidity among very-low-birth-weight (VLBW<1500g) infants in Neonatal Intensive Care Units (NICU) ***(Hornik et al., 2012).***

- **Necrotizing enterocolitis (NEC):** Necrotizing enterocolitis is the most common serious gastrointestinal disorder affecting very preterm or very low birth weight. The risk is inversely proportional to gestational age and weight at birth. Fetal growth restriction and compromise may be additional specific risk factors ***(Patel and Shah, 2012).***
- **Intraventricular hemorrhage (IVH) and periventricular leukomalacia (PVL):** The most significant forms of Perinatal brain injury observed in premature infants are intraventricular hemorrhage (IVH) and periventricular leukomalacia (PVL). IVH refers to bleeding within the ventricles of the brain, which, in severe cases, may extend into the surrounding parenchyma. The bleeding can destroy cerebral tissue and, in some cases, lead to post-hemorrhage hydrocephalus. A recent study found that infants with severe IVH have a 28 to 37 percent mortality rate **(*Brouwer et al., 2008).***
- **Bronchopulmonary dysplasia (**BPD): Bronchopulmonary dysplasia (BPD), the most common chronic lung disease in infancy, is mostly seen in infants of <30weeks gestational age and /or < 1000g birth weight**.** The lungs are characterized by fewer and larger simplified alveoli that are associated with dysmorphic vasculature. Genetic and environmental factors are involved in the pathogenesis of new BPD ***(Bhandari and Bhandari, 2011).***
- **Acute renal failure (ARF):** Acute renal failure has high mortality rate in preterm infants. There is also evidence that preterm birth adversely affects nephrogenesis (the formation

of nephrons) in the developing kidney. Certainly, there are many studies linking a reduced nephron endowment early in life with hypertension and vulnerability to secondary renal insults in adulthood ***(Hoppe et al., 2007***). In this regard, there is substantial recent epidemiological evidence linking preterm birth with an increase in blood pressure in adulthood ***(Keijzer Veen et al., 2010 b).*** ARF is an defined as an absolute increase in serum creatinine of more than or equal to 0.3mg/dl (≥26.4umol/L), a percentage increase in serum creatinine of more than or equal to 50% (1.5 fold from baseline), or a reduction in urine output (documented oliguria of less than 0.5ml/kg per hour for more than six hours)**.** Currently, abrupt (within 48 hours) reduction in kidney function measured by a rapid decline in glomerular filteration rate ***(Keijzer Veen et al., 2010 b).***

- **Congenital heart disease (CHD):** As the overall survival rate has improved for infants with congenital heart disease (CHD), there have been increased efforts made to improve long term morbidity in the context of coexisting risk factors ***(Marino et al., 2012).*** Approximately 16% with chronic lung disease (CLD) are born prematurely, and CLD is twice as common in preterm compared with term neonates. Multiple studies have documented higher rates of mortality and morbidity in neonates with CLD who are born prematurely or at lower birth weight ***(Cheng et al., 2011).***

Table (1): Frequency of congenital heart diseases

Heart Defects	*Frequency of diagnosis*
Hypoplastic left heart syndrome	5
Ebstein anomaly	2
Coarctation of the aorta	3
Transposition of the great arteries	2
Arterial septal defect, ventral septal defect,	8
Patent ductus arterious requiring surgery	8
Double-outlet right ventricle	1

Paquette LB, Wisnowski JL, Ceschin R, Pruetz JD, Detterich JA. (2013): Abnormal cerebral microstructure in premature neonates with congenital heart disease. AJNR Am J neuroadiol; 34(10) : 2026-33.

- **Hemorrhagic Disease of Newborn (HDN):** In the past, the term hemorrhagic disease of the newborn was used to describe bleeding disorder in neonates associated with a traumatic birth or hemophilia ***(Victoria, 1997).*** The proper diagnostic term that has been is currently vitamin K deficiency bleeding because vitamin K deficiency is not the sole cause of hemorrhagic disorders in preterm term infants. Although some controversy surrounds postnatal timing of the initial hemorrhage, vitamin K deficiency bleeding is usually classified by 3 distinct time periods after birth as follow ***(Pichler and Pichler, 2008):***
  - Early-onset vitamin K deficiency bleeding: Usually occurs during 24 hours. It is seen in infants born to mothers taking anticonvulsant or antituberculous medications that their actions is not clearly understood.

- Classic vitamin K deficiency bleeding: Usually occurs after 24 hours and as late as the first week of life. It is observed in infants who donot received prophylactic vitmin K. The incidence of classic vitamin K bleeding ranges from 0.25-1.7 cases per 100 births.
- Late onset vitamin K deficiency bleeding: Usually occurs between age 2-12 weeks; however, late onset vitamin K deficiency bleeding cab be seen as long as 6 months after birth. This disease is most common in breastfed infants who did not receive vitmin K prophylaxis at birth. Vitamin K is low in mature human milk and ranges from 1-4mcg/L.More than half of these infants present with acute intracranial hemorrhagic ***(Pichler and Pichler, 2008).***
- **Hyoxic Ischemic Encephalopathy (HIE):** Perinatal asphyxia, more appropriately kown as hypoxic –ischemic encephalopathy (HIE), is characterized by clinical and laboratory evidence of acute or subacute brain injury due to asphyexia. The primary causes of this condition are systemic hypoxemia and / or reduced cerebral blood flow (CBF). Birth asphyxia causes 840.000 or 23% of all neonatal deaths worldwide ***(Ferriero, 2004).***

  Symptoms & Signs of HIE: Differentiated into mild (HIE) in it baby may be slightly increase muscle tone, moderate (HIE) in it baby is lethargic with significant hypotonia, severe (HIE) in it seizures can be delayed and severe and may be initially resistant to anticonventional treatment.

  Diagnosis of HIE: Laboratory (as serum electrolytes, renal fn., cardiac and liver enzymes, coagulation system

and arterial blood gases) and imaging studies (as MRI of brain, cranial u/s and ECG) ***(Zanelli, 2014).***

BRONCHOPULMONARY DYSPLASIA (BPD)

A number of definitions of bronchopulmonary dysplasia (BPD), or chronic lung disease, have been used.

Old definition of BPD:

Bronchopulmonary was first described as lung injury in preterm infants resulting from oxygen and mechanical ventilation ***(Northway et al., 1967).***

Bronchopulmonary dysplasia (BPD): it is currently defined as the need for O2 supplementation for 28 days of life and a 'physiologic 'assessment of the O2 at 36 weeks' postmenstrual age ***(Bhandari and Bhandari, 2011; Trembath and Laughon, 2012).***

Modified definition of BPD (New definition of BPD):

Currently the description of BPD includes the grading of its severity into mild, moderate and severe. This correlates with the infant' s maturity, growth and overall severity illness.

Mild and moderate BPD are defined as a need for supplemental oxygen ≥ 28 days, but not at 36 weeks' Postmenstrual age (PMA), while severe BPD is defined as a need for supplemental oxygen at 36 weeks, postmenstrual age (PMA), but not requiring ventilation assistance, and a classification of very severe BPD required a need for both supplemental oxygen and ventilation assistance at 36 weeks PMA ***(Sriram et al., 2014).***

Despite many advances in neonatal ventilation technigues, widespread use of surfactant and antenatal corticosteroids as

well as aggressive fluid management, BPD is associated with significant pulmonary and neurodevelopmental sequalae that continue to have health ramification into adulthood ***(Anderson and Doyle, 2006; Bhandari and Panitch, 2006; Bhandari and Bhandari, 2011).***

Bronchopulmonary dysplasia (BPD) is the presence of respiratory signs and symptoms, the need for supplemental oxygen to treat hypoxemia, and an abnormal chest X-ray at 36weeks postmenstrual age (gestational age plus chronological age) ***(Kinsella et al., 2006).***

Diagnostic criteria of Bronchopulmonary dysplasia:

- Mild:
  - Breathing room air at 36 weeks' post-menstrual age or discharge (which comes first) for babies born before 32 weeks, or
  - Breathing room air by 56 days' postnatal age, or discharge for babies who born after 32 weeks gestation.
- Moderate:
  - Need for < 30% oxygen at 36 weeks postmenstrual age, or discharge for babies born before 32 weeks. or
  - Need for < 30% oxygen at 56 days postnatal age, or discharge.
- Severe:
  - Need for > 30% oxygen, with or without positive pressure ventilation or continuous positive pressure at 36 weeks