

Chapter Two

Materials and Methods

2.1 Study design:

This is a case control analytical study conducted in Omdurman Maternity Hospital , Khartoum State during the period (April-July) 2015 .

2.2 study population :

One hundred neonates with confirmed neonatal sepsis (case) and thirty healthy neonates (control) of both sexes were enrolled in the study.

2.3 Inclusion criteria:

Full term septic neonates aged less than 7 days and free from any disease.

2.4 Exclusion criteria :

Preterm neonates , neonatal age more than one week, were excluded . Neonates having other diseases and major congenital malformation were excluded.

2.5 Data collection :

Designed questionnaire was used to collect basic and clinical data from the mothers.

2.6 Sample collection :

Venous blood 1 ml was collected from each neonate into a vacutainer tube containing ethylenediamine tetra acetic acid (EDTA) as an anticoagulant using disposable needle and plastic holder , each sample was mixed gently to prevent cell lysis and to ensure mixing with the anticoagulant .

2.7 Materials and methods :

2.7.1 General Equipment:

Automated hematological analyzer (Sysmex KX2IN) was used for complete blood count,

2.7.2 Principle of sysmex KX2IN :

Sysmex measurement of blood cells (WBCs, RBCs,, HB ,Hematocrite(PCV) ,mean corpuscular volume (MCV) mean corpuscular hemoglobin , mean corpuscular hemoglobin concentration (MCHC),Plts count and differential leucocyte count.) by aspiration of a volume of well mixed EDTA blood by prob and mixed with isotonic diluent in nebulizer. Diluent mixture aspiration delivered to RBCs aperture path for providing information about RBCs and Plt based on size .Particles of 2-20 fl counted plt above 36 fl counted as red cell ,some portion of aspirated mixture induced into WBCs path in which hemolytic reagent (stromatolyzer) was added automatically to measure HB concentration in a build calorimeter , based on cynomet hemoglobin (HiCN) information generated in triplicate pulses according to electronic conductivity and translated into digital number using in build calculator programmed and deigned for RBCs , WBCs count hence three values were directly measured (RBCs, WBCs, HB)and display on (LCD) ,other values fpr red cell indices .Plt ,leucocyte differential and absolute count calculated from given information and automated constructed histogram. The result printed out according to setting mode.

2.7.3 Reagents :

Cell back(diluent) :

Active ingredient : sodium chloride , boric acid , sodium tetra borate .

Stromatolyzer :

Active ingredient : organic ammonium salt , non-ionic surfactant.

Sulfolizer :

Active ingredient : Sodium laurylsulphate

Cell clean :

Active ingredient: Sodium hypochloride.

2.8 Ethical consideration :

Ethical approval from Sudan University of Science and Technology and from Omdurman Maternity Hospital weretaken .Verbal consent from the mothers was taken at the beginning of the study , they were assured that the results will not be used for anyother purpose than this study, and will be kept confidential .

2.9 statistical analysis :

Data were analyzed by SPSS program used for data entry analysis . Independent T-test were conducted to calculate mean of both case and control and chi- square test for correlation between risk factor and sepsis , using SPSS computer version 20.