# Identification of granulopoiesis lineage in bone marrow of adult male albino rat "Rattus norvegious".

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Key word: *Granulopoiesis , Bone marrow*.

**(Received :May 2014, Accept : Jun 2014)**

## Abstract

Bone marrow becomes increasly important hematopoietic tissue. The stroma of the bone marrow is composed of hematopoietic cords, sinusoids, and collagen fibers. The earliest recognizable of granulocytic lineage is the myeloblast. Myeloblast is an oval cell and its chromatin distributed within the nucleus. The promelocyte is sometime slightly larger that its progenitor. Its cytoplasm contains a variable number of large coarse granules. The metamelocytes become smaller and their nucleus attains a definitive indentation. The band cell has a nuclear indentation with concentrate and course chromatin. The segmented cells are characterized by a multilobulated nucleus showing a densely packed chromatin with some clumping.

## دراسة تحديد مراحل نضج الخلايا الحبيبية في نقي عظم ذكر الجرذ الابيض"Rattus norvegious"

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**مفتاح الكلمات**: مراحل نضج الخلايا الحبيبية, نخاع العظم

**الخلاصة**

يعد نقي العظم , نسيج دموي مهم. يتكون متن نقي العظم من حبال دموية وجيبانيات شعرية والياف غراوية. ان الخطوة الاولى في تكوين الجيبانيات تبدا في الارومة النقية اذ تكون خلية بيضوية وينتشر الصبغين في نواتها.تكون الخلية قبل الحبيبية اكبر قليلا من الارومة النقية ويحتوي هيوليها على حبيبات خشنة , تكون الخلية بعد النقية صغيرة الحجم ونواتها مسننة , تكون نواة الخلية الشريطية مسننة وذات حبيبات صبغينية مركزة. تمتاز الخلايا الشدفية بنواتها المتعددة الفصوص وذات صبغين مظغوط ومتجمع.

## Introduction

Adult mammals, granulopoiesis occurs primarily in the bone marrow with expansion to extramedullary sites in times of increased demand or with bone marrow disease (1, 2). The various granulocytic lineages in adult mammals originated from pleuripotential hematopoietic stem cells within the bone marrow. Hematopoietic stem cells reside in close association with osteblast along the endosteal border of bone marrow (3,4). Proliferation, mobilization, and commitment hematopoietic stem cells to granulocytic differentiation occur in proposed vascular niches along the vascular sinuses of the central bone marrow (5). The granulocytic lineages differentiate from common myeloid progenitor to produce neutrophil progenitors, eosinophil progenitors, basophile progenitors and mast cells progenitors. Basophile and mast cells progenitors subsequently give rise to basophile progenitors, which complete maturation in bone marrow (6, 7, 8 ). The aim of this research is to study granulopoiesis of bone marrow in rats as vast information concerning hematopoietic cell exists, however, less attention has been paid to bone marrow granulopoiesis.

## Materials and methods

Twenty clinically healthy adult male rats were used in this study. These animals weighting 320-410 gm ±5 gm and have four to five months old. They were given a rodent diet supplement with vitamin C, and supplied with water adlibtum.

Each animal was general anesthetized with ketamine (25-40 mg/kg) and zylazine (5-12 mg/kg) given I/M (9). In these animals, bone marrow is obtained by aspiration of the femur bone and iliac crest. Bone marrow spread between the slides and the smear are rapidly air dried. These prepared slides are dipped into acetic acid at 4C° for 24 hours and then dipped into methanol for less than 10 minutes to remove any debris or fat. Staining with romanwesky-type blood stains such as Giemsa stain and Wright stain and diff-quik stain. Photograph of examined slides were carried out with amotic microscope which is supplied with a digitals camera with a resolution power of five mega pixel.

## Result

The first granulocytic lineage during the process of granulopoiesis beings with the myeloblast. Myeloblast is easily recognizable by light microscope (Fig.1). The myeloblast is characterized by finely stippled. Chromatin within its nucleus. The eccentric round or oval nucleus contains one or multiple nucleoli, and moderately basophilic cytoplasm, its cytoplasm has small numbers of granules. Myeloblast is usually a large cell. The promyelocyte (progranulocyte) appears larger than the myeloblast (Fig.2a,b,c). Promyelocyte is characterized by increasing numbers of granules and decreasing cytoplasmic basophilia. The nucleus is slightly smaller and the chromatin pattern remains finely stippled and nucleoli may or may not be present. The myelocyte (Fig.3) is smaller than promyelocyte and is characterized by a round to slightly oval nucleus with partial condensed chromatin and lightly basophilic cytoplasm containing granules. The metamyelocyte (Basophilic metamyelocyte, Eosinophilic metamyelocyte, Nutrophilic metamyelocyte) undergo nuclear maturation characterized increasing condensation of chromatin and progressive segmentation of the nucleus. The metamyelocyte has been shaped to elongate nucleus. Metamyelocyte are divided into basophilic metamyelocyte, eosinophilic metamyelocyte, nutrophilic metamyelocyte (Fig.4 a,b,c). The cytoplasm of the nutrophilic

metamyelocyte is pale-yellow in colour or appears colourless with condensed granules. The cytoplasm of the eosinophilic metamyelocytes is usually a little bluer than nutrophilic metamyelocytes and posses spherical granules. The granules of the basophilic metamyelocyte appear diffused or ill-define. All the constituents of basophilic metamyelocyte being purple-red in colour. The band cells between (Stab cells, non-segmented cells) represent the intermediate cells between metamyelocyte and mature segments (Fig.5). The shape of the nuclear band cell is often in the form of a circle, sometimes in the form of the sinuous or twisted and lies nearer to the periphery of the cell. The nuclear chromatin is more condensed. Band cell appears as nutrophilic band cell, eosinophilic band cell, basophilic band cell (Fig.6, 7, 8).

The segmented cell (Nutrophile, Eosinophile, Basophile segmented cells). The cytoplasm of Nutrophile segmented cell was pale and contained indistinct granulation. The eosinophilic segmented cell appears to be slightly larger than nutrophilic segmented cell. The cell posse's irregular nucleus which constricted in to lobes and has heavily clumped nuclear chromatin. The basophilic segmented cell can be identified by its larger and darker staining granules which evenly scattered throughout the cytoplasm (Fig.9, 10, 11).

**Discussion**

The cells of the blood were originated from the bone marrow. The active is confined to selected areas of the bones in the end and the shift of the long bone, the flat bones, head, pelvis, ribs and the vertebral bodies (10,11). Bone marrow can be divided into two broad compartments; the hematopoietic cells, and the stromal cells. The regulations of the mammalians hematopoiesis depend on a microenvironment which composed of a variety of hematopoietic cells, stromal cells, and their extracellular matrix (12, 13). Granulopoiesis is regulated by movement of hematopoietic precursor cells through niches in bone marrow in response to a series of factors produced by cells in the microenvironment, interaction with extracellular matrix, and factors delivered from the blood (14). The lineage of the granulocyte development in order of differentiation include firstly the myeloblast which has ovoid nucleus with two or more nucleoli. Its cytoplasm is basophilic. The diversity of research noted that in the pathological conditions, the myeloblast may become more numerous and may appear in considerable numbers in the circulating blood as in myelogenous leukemia(15). Promyelocyte posses round or oval nucleus, occasionally indented. The basophilic cytoplasm containing azurophilic granules. In contrast (16) in which the reports indicate that the nucleus of the promyelocyte was slightly smaller and contain no nucleoli. Myelocyte can be distinguished into nutrophilic myelocyte, acidophilic, and basophilic myelocyte posses granules which vary greatly in shape, size, and concentration in domestic species as declared by some worker (17). The nuclei of metamyelocyte appears irregular in shape and this cell also present in three types. The observation resercher believed that the cytoplasmic granules of metamyelocyte contain histamine, heparin, bactericidal lactoferrin and acid mucopolysaccharide (18). Band cells appear in three series also. Their nuclei vary in shape from U,C,S kidney shapes that coincide with (19). The segmented cell has a segmented nucleus that form 3-5 nuclear lobe joined by chromatin filament. This study is in agreement with (20).

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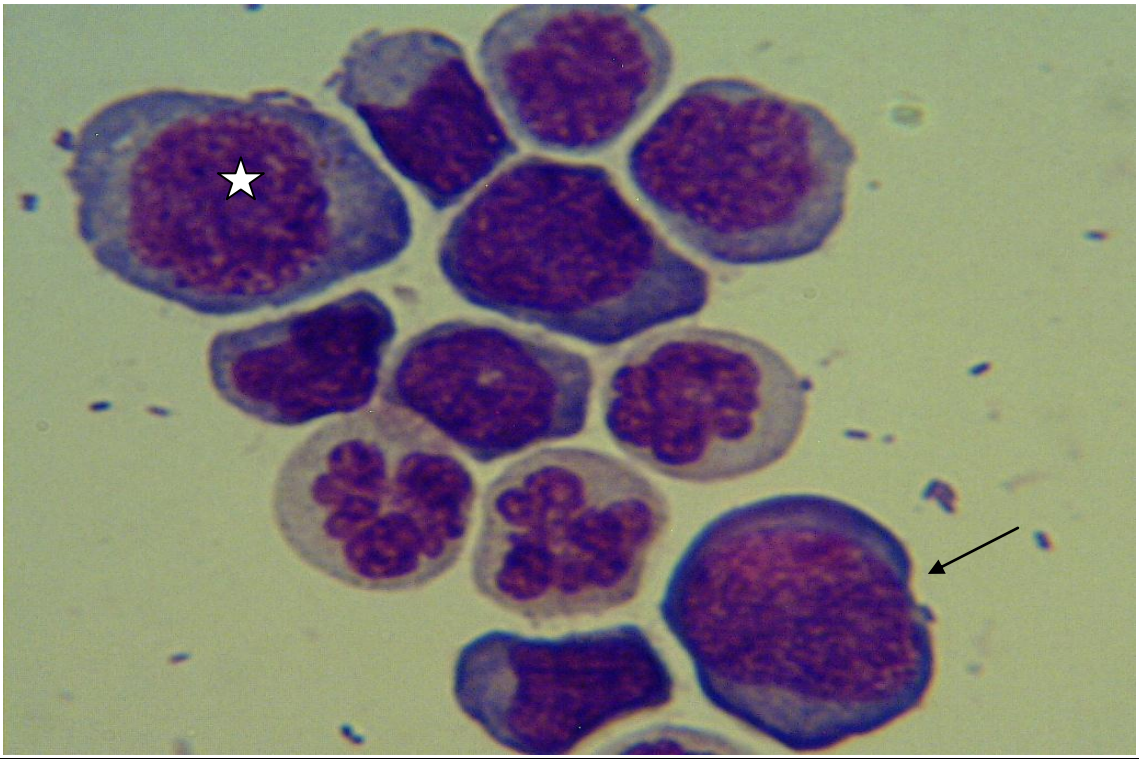

Fig.1 Myeloblasts a large cell, and characterized by a deeply basophilic cytoplasm (star) Promyelocytes arrow Giemsa stain X100

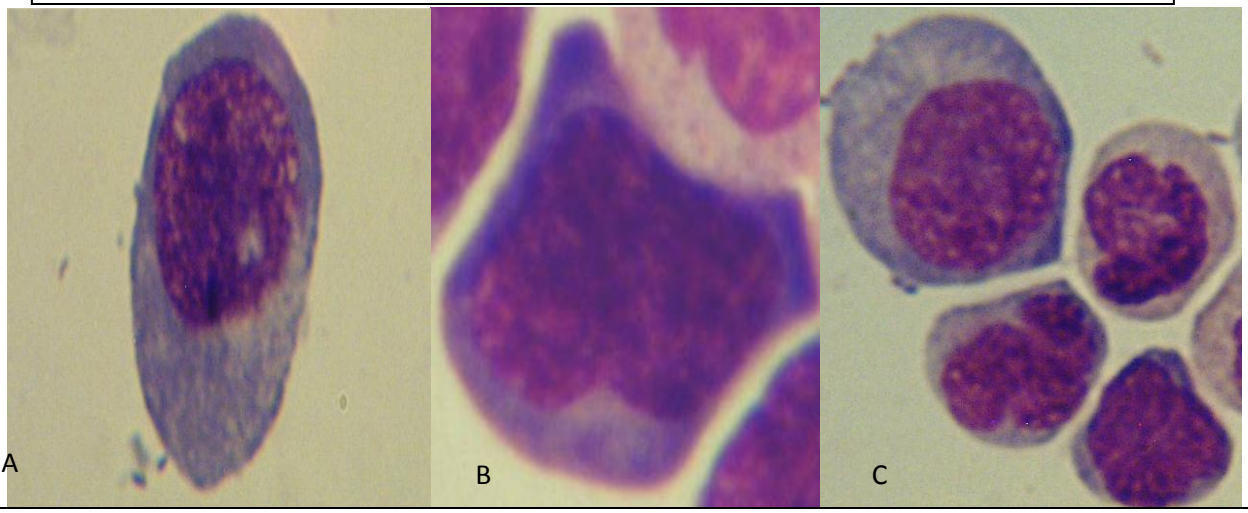


Fig.2a Neutrophilic Promyelocytes are large cells, coarser chromatin, and abundant basophilic cytoplasm containing purple or azurophilic

A-Giemsa stain B-Diff quick C-Wright stain X100

Fig.2b Eosinophilic Promyelocytes (arrow), eosinophilic granules presence in the cytoplasm. Eosinophilic metamyelocytes (star) Giemsa stain X100

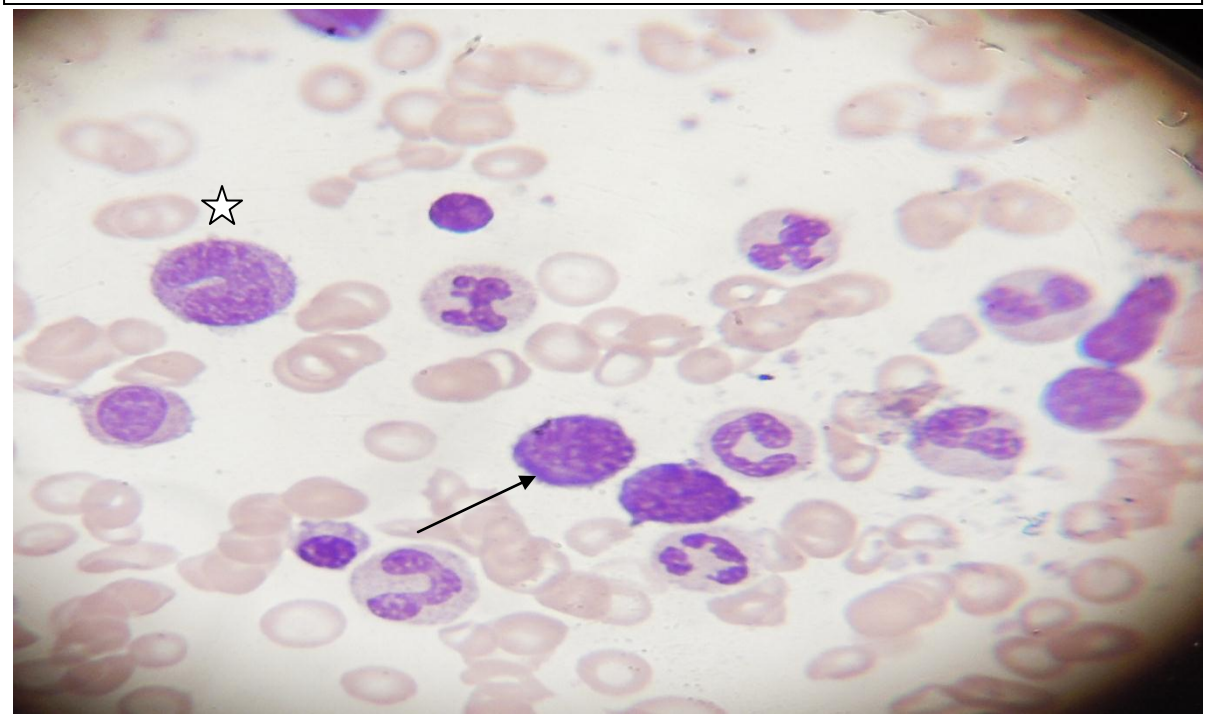

Fig.2c Basophilic Promyelocytes (arrow), Basophilic granules presence in the cytoplasm. Basophilic metamyelocytes (star) Wright stain X100

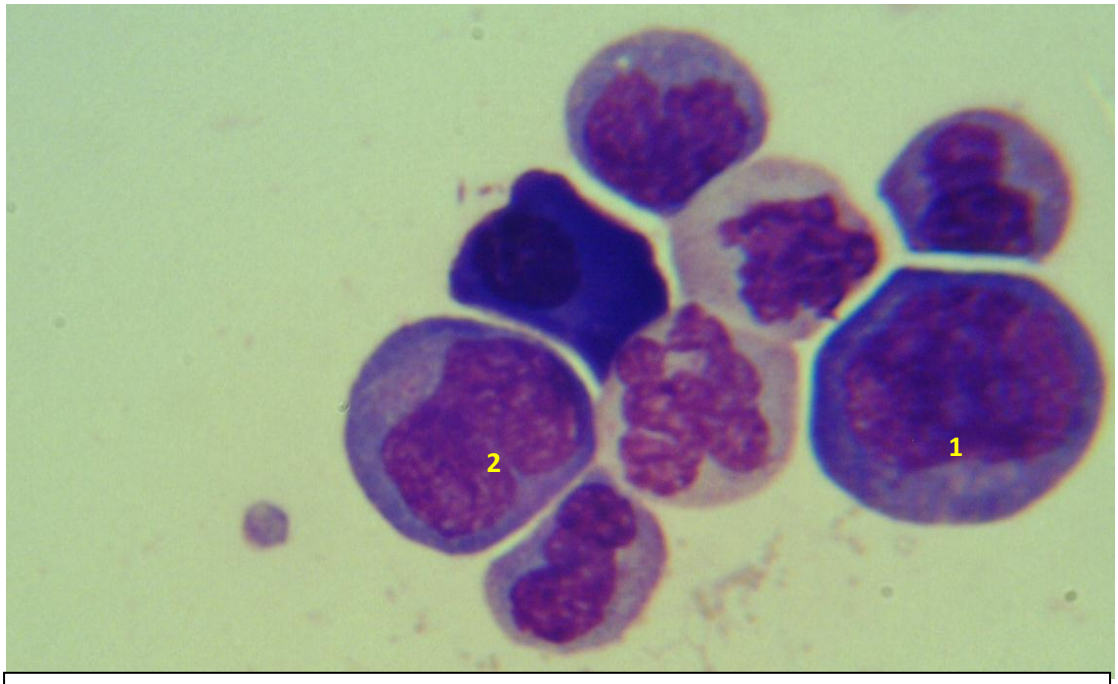


Fig. 3 granulocyte myeloid series 1-myeloblast 2-promyeloblast Giemsa stain X100

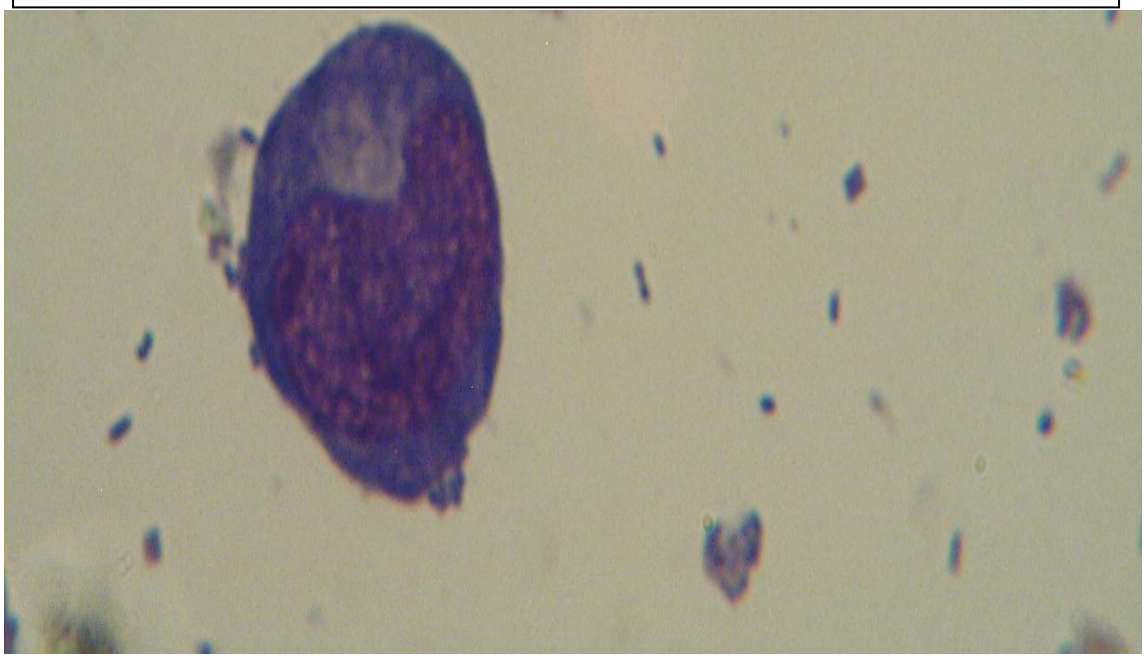

Fig. 4a Neutrophilic Metamyelocytes Diff quick stain X100

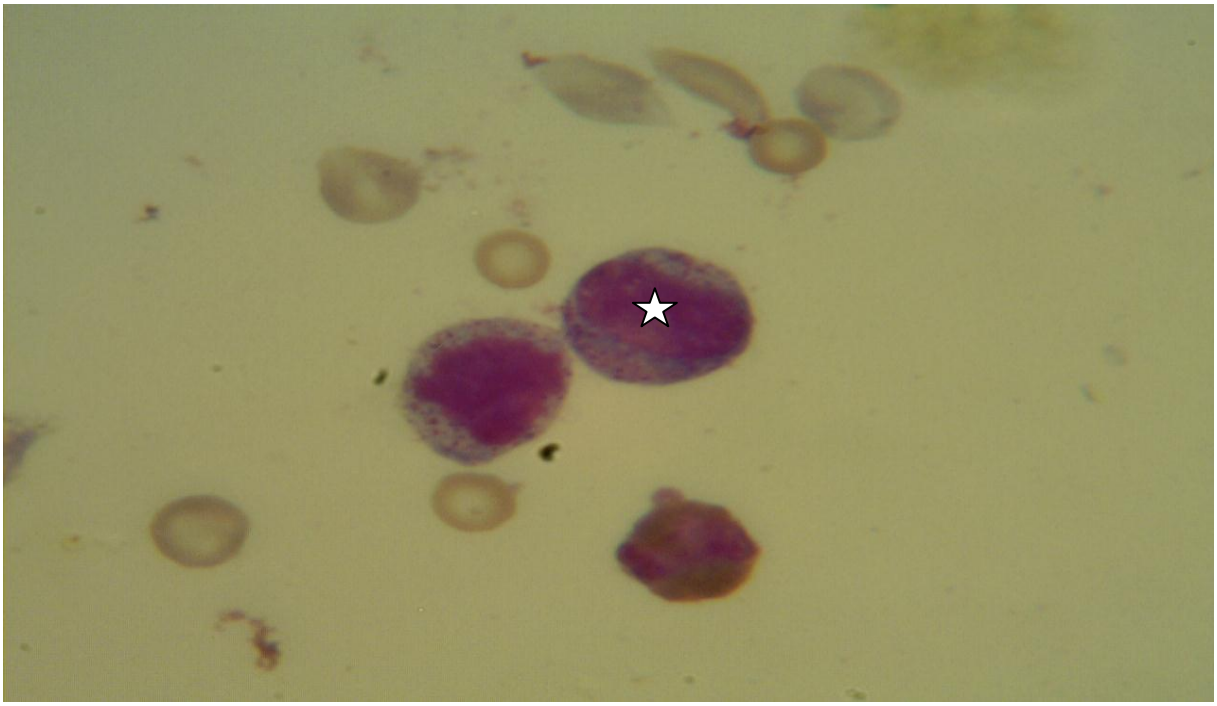

Fig.4b Eosinophilic Metamyelocytes (star) Giemsa stain X100

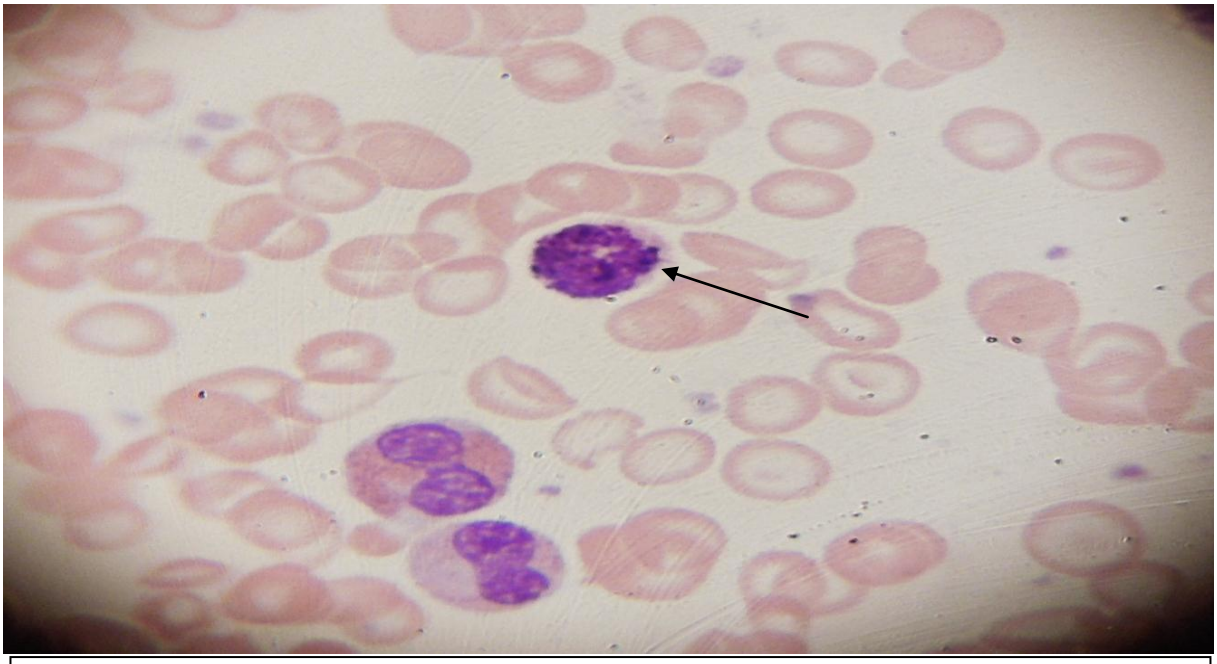

Fig. 4c basophilic metamyelocytes (arrow), Eosinophilic Metamyelocytes (star). Giemsa stain X100

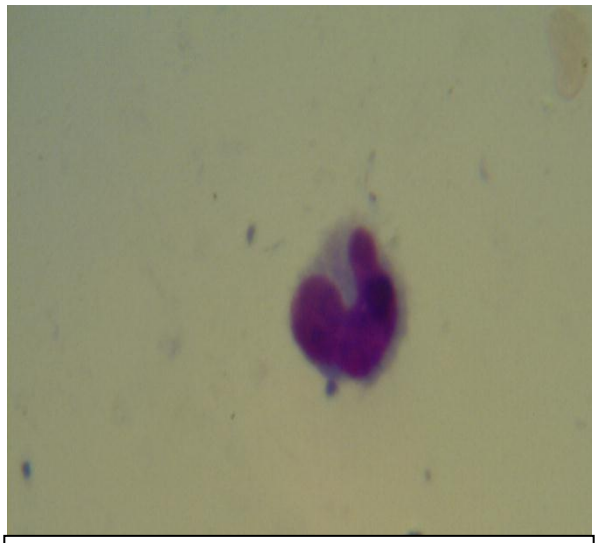

Fig. 5 Early band cell Giemsa stain X100

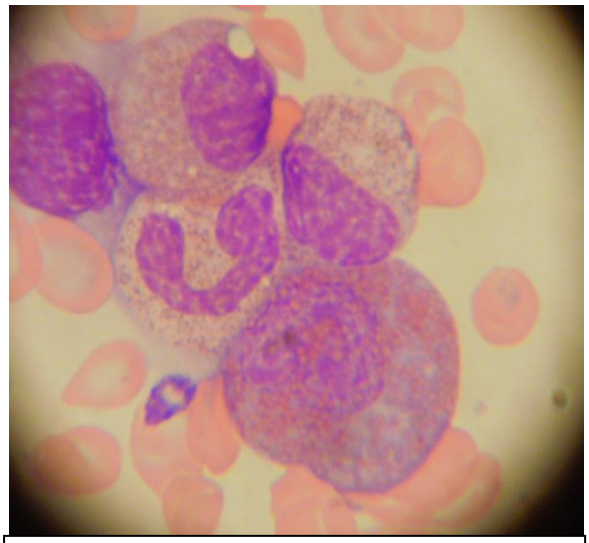

Fig. 6 Nutrophilic Band cell (arrow) Wright stain X100

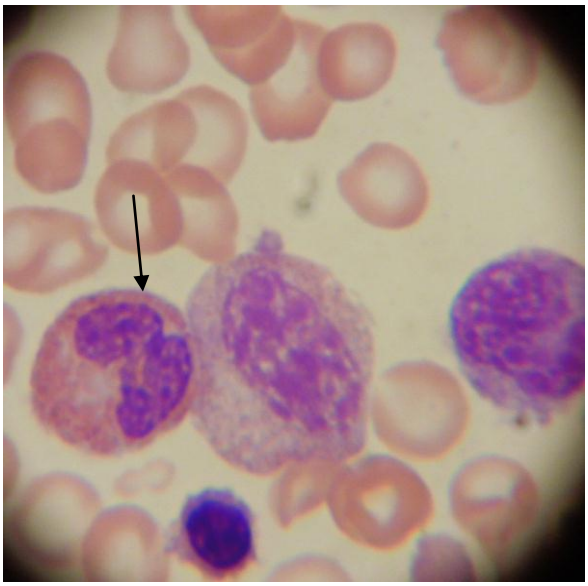

Fig. 7 band eosinophile (arrow) Wright stain X100

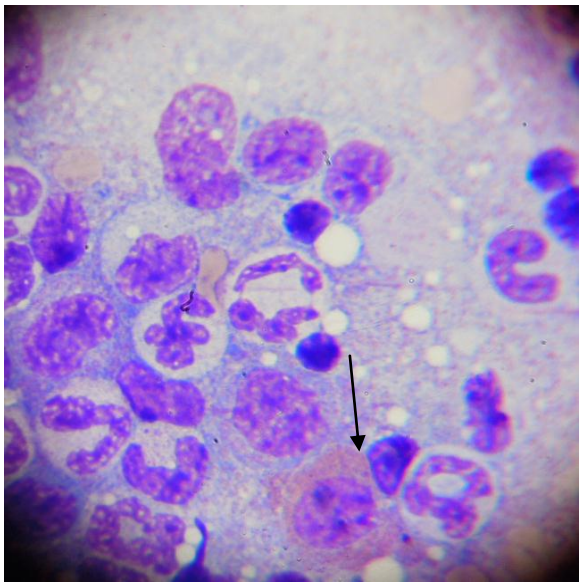

Fig. 8 band basophiles (arrow) Wright stain X100


Fig.9 A-Late Band neutrophil B-early band neutrophil C- segmented neutrophil Giemsa stain X100

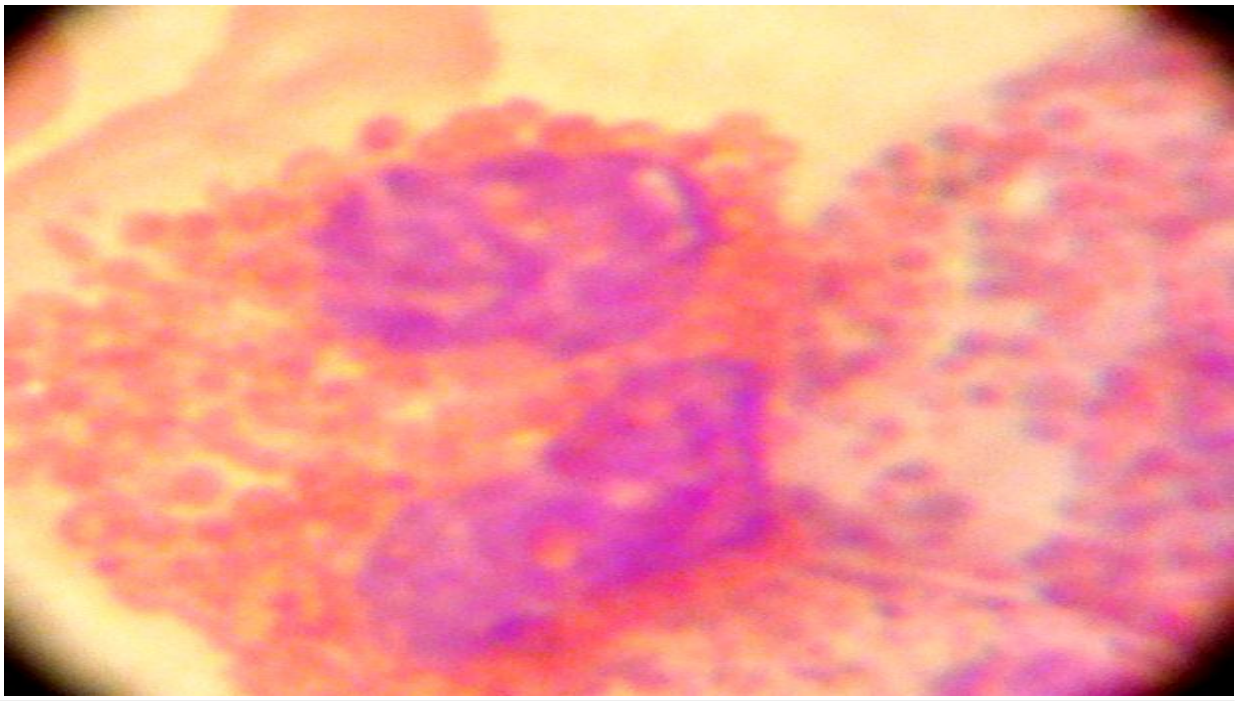

Fig.10 segmented eosinophils has a bilobed nucleus and its cytoplasm is filled with large eosinophilic granules X 100 Wright stain

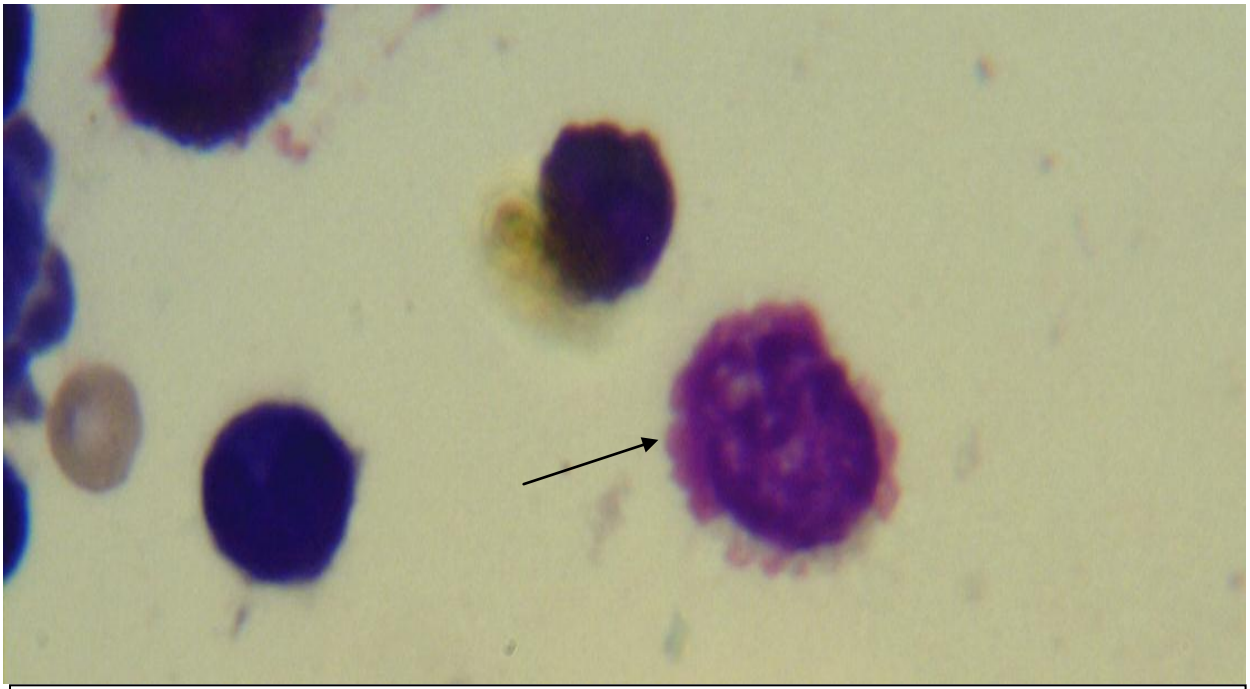

Fig.11 Basophilic granulocytes (arrow) Giemsa stain X100