Original Article

Glycogen Accumulation in Neutrophil Can Be a Marker of Sepsis

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Abstract

Background: Sepsis, a systemic inflammatory reaction to infection, is the leading cause of death in the world. The early detection and identification of pro-inflammatory changes in overall metabolism and functioning can help in the proper intervention and control of the inflammatory state, and it will improve the prognosis. **Aim:** In a resource-limited setting where the biomarkers are not easily accessible, this simple technique is required that can help in the early identification of infection and inflammation. **Methodology:** The present study was conducted to find the change in glycogen accumulation and morphological changes during inflammation by preparing a peripheral smear and periodic acid–Schiff (PAS) staining. **Results:** This study shows that the neutrophil accumulates glycogen granules throughout the cytoplasm with the presence of vacuoles in the cytoplasm, thus increasing the neutrophil size and chromatin dispersion. **Conclusion:** PAS staining can be used as a diagnostic method to detect sepsis.

Keywords: *Glycogen granules, glycolysis, periodic acid–Schiff staining, sepsis, Warburg effect*

Introduction

It has been more than 90 years since the term "Warburg Effect" entered our scientific lexicon. Warburg effect highlights, for proliferation and biosynthesis of tumor cells, rely on glycolysis.[1] During activation, monocyte, macrophage, dendritic cell, and T-cell follow the same mechanism.[2] In mammals and eel, glycogen content increases in inflammatory polymorphonuclear leukocytes (PMNs) as compared to normal.[3-5] The inflammatory PMNs have ten-fold more glycogen than blood PMNs.[4] The cells of myeloid lineage derive their energy almost exclusively from glycolysis during inflammation. Even proliferating lymphocytes depend on glycolysis for energy.[6] Consistent with the above line of thinking, changes like an accumulation of glycogen may be the early marker of sepsis and infection.

Sepsis, a systemic inflammatory reaction to infection, is the leading cause of death in the world. C-reactive protein, procalcitonin, interleukin-6, etc., are sepsis biomarkers usually used along with white blood cell count, their changes in morphology, metabolism, the presence of inclusions glycogen, and lipid granules for detection of sepsis. The metabolic by-products such as lactic acid can also be easily detected, and levels were evaluated to know the extent of infection and inflammation. In a resource-limited setting where the biomarkers are not easily accessible, this simple staining technique can help in the early identification of infection and inflammation. The early detection and identification of pro-inflammatory changes in overall metabolism and functioning can help in the proper intervention and control of the inflammatory state, and it will improve the prognosis.

Methodology

A case–control study for studying the increase of glycogen granules in blood cells was carried out in a tertiary care hospital in Bhubaneswar, Odisha, India, from May 2016 to August 2016. Prior approval of the Institutional Ethics Committee was obtained before commencing the study. The participants who were agreed to sign the informed consent form were included in this study.

Cases were the participants aged between 18 and 60 years and who were diagnosed with sepsis in the tertiary care hospital. Consent was obtained from them or their family members for participation in the study. The participant who was confirmed as sepsis patient was considered for this

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study. Criteria used were (any two of the four) as follows: body temperature >38.5°C or <35.0°C; heart rate >90 beats/min; respiratory rate >20 breaths/min or need for mechanical ventilation; white blood cell count >12,000/mm^3 or <4000/mm^3 or immature forms >10%. Finally, blood culture-positive participants were considered for the study.

Controls were employees and workers of the same age group in the tertiary care hospital who were contacted personally or through E-mail and requested for participation in the study. Apparently healthy participants who were not suffering from any diseases were chosen on first come, first served basis and registered after obtaining a written consent until the required numbers were obtained.

The participants who were confirmed as sepsis patients and fulfilled the inclusion criteria were considered as cases, and age-matched healthy participants were considered as controls.

Blood smears were made on the glass slide. Periodic acid–Schiff (PAS) staining was used for the demonstration of glycogen. Periodic acid oxidizes the blood film, resulting in the formation of aldehyde grouping. The aldehyde groups are then detected by the Schiff base. Air-dried blood films were fixed for 1 min at room temperature in formalin–ethanol fixative solution and then rinsed for 1 min in slowly running tap water. After that, slides were immersed in periodic acid solution for 5 min at room temperature followed by rinsing of slides in several changes of distilled water. Then, slides were immersed in Schiff's reagent for 15 min at room temperature. It was followed by washing in running tap water for 5 min. Counterstaining was done in hematoxylin solution for 90 s. Slides were again rinsed in running tap water for 15–30 s after counterstaining and air-dried and were examined microscopically under oil immersion lens, in toluene- or xylene-based mounting media.

Statistics

Sensitivity and specificity tests were done for intracytoplasmic granules' findings between cases and control results. Student's *t*-test was applied for neutrophil diameter. *P* value < 0.05 was considered significant.

Results

Neutrophil diameter increased in cases as compared to controls. Similarly, number of glycogen granules were >77 on an average in cases as compared to nil in controls [Table 1].

Of the 18 sepsis patients we examined, all were true positive for intracytoplasmic neutrophilic granules, and there were no false-negative cases. Similarly, of 16 controls, all were found to be true negative for intracytoplasmic granules. Hence, the sensitivity was found to be 100%, and the specificity was also found to be 100%. The positive predictive value and negative predictive value for this test were found to be 100%.

Table 1: Neutrophil diameter and glycogen granules in neutrophil of cases and controls

Neutrophil	Controls (average)	Cases (average)
Diameter (μm)	11.76044±1.661174	19.85844±1.194265
Number of glycogen granules	No granules	77.18342±7.757359

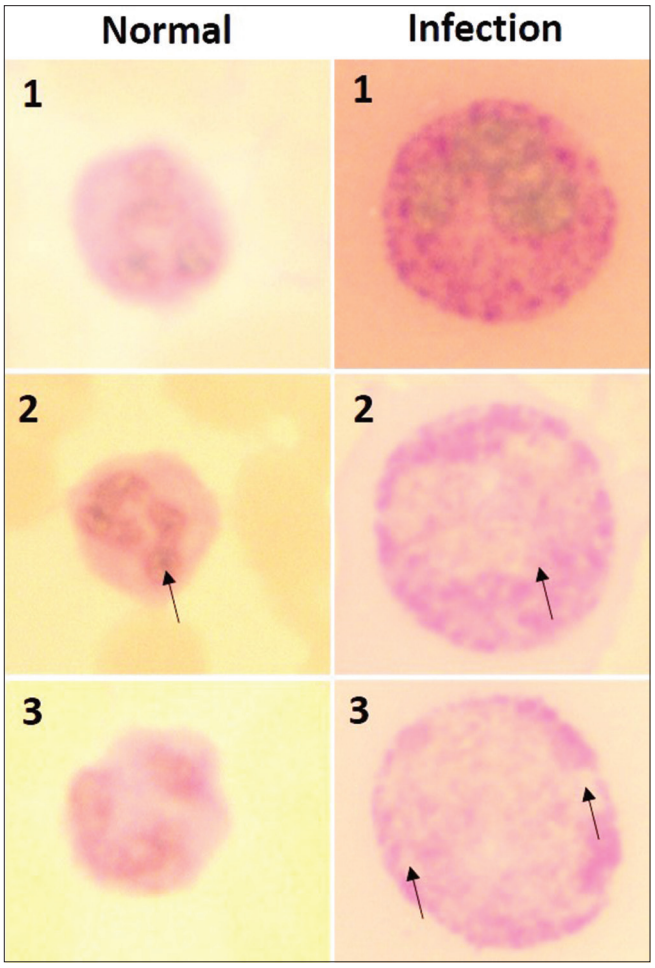

Figure 1: (1) Abundant periodic acid–Schiff-positive granules, (2) relaxed chromatin, (3) vacuole and increased neutrophil size in infected patients as compared to normal individuals

Discussion

PAS-stained neutrophils examined by light microscopy revealed clear vacuoles within a significant number of neutrophil from an infected patient. Such vacuoles were not seen in control neutrophils from a healthy individual. Visually detectable, dramatic increases in the neutrophil diameter were observed in infected patients compared healthy individuals. Compared to controls (11.76044 ± 1.661174 μm), the diameters of neutrophil in the infected patient are large (19.85844 ± 1.194265 μm). The size of neutrophil in noninfected individual was homogeneous. Dispersed chromatin and the presence of nucleolus were very frequent in neutrophil from infected patients, whereas chromatin

condensations are clearly invisible in neutrophil from healthy individuals [Figure 1].

Conclusion

In the case of infection like sepsis, the increase of glycogen granules and other additional changes may be a useful biomarker for rapid and economical diagnosis. Early awareness of the patient condition from the abovementioned marker may be the future instrument for providing better patient care in a resource-limited setting.

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Conflicts of interest

There are no conflicts of interest.

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