



**MONITORING EFFICACY OF BENZYL PENICILLIN IN DIPHTHERIA PATIENTS  
BASED ON VALIDATED HEMATOLOGICAL ANALYZER IN HODEIDAH  
OUTBREAK, YEMEN**

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**ABSTRACT**

**Background:** Benzylpenicillin (penicillin G) is the chemotherapeutic agent that uses to treat the diphtheria, monitoring efficacy of benzylpenicillin takes many forms (monitoring of antibiotic concentration, monitoring of laboratory tests, monitoring of biochemical markers as a response to treatment and /or toxicity, and monitoring clinical response to treatment. **Objective:** Therefore, the study aims to validate laboratory test namely hematological analyzer for monitoring the efficacy of benzylpenicillin for diphtheria treatment in Hodeidah outbreak, Yemen, 2018. **Materials and Methods:** The hematological analyzer (Sysmex KX-21) was validated by assessment of the linearity, accuracy, precision and quantification limit of different blood samples and used for samples research analysis namely monitoring the efficacy of benzylpenicillin. 90 patients in the study were provided by informed consent. The patients were diagnosed with diphtheria according to national standard and they received benzylpenicillin 50 IU / kg daily for two weeks. The white blood cell (WBC) and related parameters namely lymphocytes, monocytes, neutrophils, basophils, and eosinophils were monitored in three phases pre treatment, within treatment, and post treatment of diphtheria patients. On the other hand, monitoring efficacy of benzylpenicillin based on in vitro assay was carried out. Obtained data were analyzed by on appropriate statistical tools. **Results:** The results of validated method were precise to each analyte with percent relative standard deviation (RSD %) of precision namely < 5.0 % for WBC and < 16.0 % for neutrophils. Furthermore, the accuracy of validated method exhibited well recovery value of (97 – 103 %) and the coefficient correlation ( $R^2$ ) value was more than 0.9995 as a good linear method. On the other hand, the results showed the level of WBC ( $\times 10^9/L$ ), and neutrophils (%) with benzylpenicillin in 77/90 cases (85.55 %) to reduce significantly from  $20.2 \pm 5 (\times 10^9/L)$ ,  $80 \pm 10$  (%) pretreatment phase into  $9.1 \pm 5 (\times 10^9/L)$ ,  $65 \pm 5$  (%) in post – treatment phase ( $p < 0.05$ ), respectively. The result of level of WBC and neutrophils in follow up phase were  $6.2 \pm 2 (\times 10^9/L)$ ,  $45 \pm 5$  (%). **Conclusion:** The hematological parameters means of this study namely WBC and neutrophils were economical indicator for monitoring the efficacy of antibiotic, where WBC and neutrophils increased with diphtheria infection and reduction with antibiotic administrated to normal rang were found. Briefly, the benzylpenicillin has effectiveness against diphtheria.

**KEYWORDS:** Monitoring, Efficacy, Benzylpenicillin, Diphtheria, Validation, Hematological.

**1. INTRODUCTION**

Monitoring takes many forms and there is evidence that in many situations it is done inappropriately (too much or too little or at the wrong time) or not targeted at specific parameters that are clinically useful. Clinicians are frequently asked to monitor the effects of drug treatment with the objective of ensuring safe and effective therapy. Monitoring of drugs includes (a) monitoring of laboratory tests that indicate to monitor the adverse effect reaction, (b) monitoring of drug concentration to

attain therapeutic response without dose related toxicity, or to confirm compliance, (c) monitoring of biochemical markers as a response to treatment and /or toxicity. (d) monitoring clinical response to treatment.<sup>[1,2]</sup>

The article describes the monitoring efficacy of benzylpenicillin (penicillin G) in terms of potency and accurate measurement of potency is critical in pharmacology to safe and proper use in diphtheria treatment according to World Health organization

(WHO).<sup>[3]</sup> The article provides a complete blood count (CBC) namely white blood cell (WBC) to monitor the efficacy of antibiotics namely benzylpenicillin as biochemical markers as a response to treatment.

The results of a CBC can provide information about not only the number of cell types but also can give an indication of the physical characteristics of some of the cells. Also, these parameters play important role to help doctors know these values in a true way so that they can come to a sound management namely diagnosis and treatment.<sup>[4-7]</sup>

This study was aimed to validate the hematological analyzer for monitoring the efficacy of benzylpenicillin in diphtheria treatment in Hodeidah, Yemen, 2018. Also, to make a economical indicators for the evaluation the effectiveness of antibiotic that is true and sound for bacterial infectious diseases namely diphtheria.

## 2. MATERIAL AND METHODS

### 2.1. Study design

This was a randomized clinical trial study about monitoring the efficacy of benzylpenicillin based on using validated hematological analyzer in Hodeidah outbreak, Yemen. The study was conducted during the period January 2018 to September 2018.

### 2.2. Study population

The personal data was collected through structured questionnaire namely diphtheria investigation form, including demographic data, vaccination status, and clinical data.

### 2.3. Study area

This study was conducted in isolation department, center of tropical medicine and infectious diseases, authority of public AL- Thawara hospital, Hodeidah selected Yemen country that is tropical region Hodeidah Governorate borders the Red Sea and is part of the narrow Tihama region. The study included all patients that were admitted in isolation department.

### 2.4. Inclusion and exclusion criteria

The inclusion criteria included the diphtheria patients, aged between 1 – 50 year old, while the exclusion criteria included the patients with other infection (coinfection) and chronic diseases).

### 2.5. Sample collection

Whole blood namely 90 (38 males and 52 females) samples were collected into EDTA anticoagulated tubes and mixed well with the anticoagulant.

### 2.6. Ethical issues

The study was integrated within the clinical practice. Patients received simple explanation for the aim of the study. If agreed to participate verbally, blood sample was collected and interview was conducted. Volunteers were reassured that this will be treated as usual.

Confidentiality of the collected data was achieved by keeping data record in a locked room with limited access to the research team only. according to university of Hodeidah and Center of Tropical Medicine and Infectious Disease (CTMID) of Authority of Public AL- Thawara Hospital, Hodeidah, Yemen declaration. Ethical, we cannot use antibiotic alone, also cannot use antitoxin. Therefore, the treatment included benzylpenicillin - diphtheria anti-toxin (DAT) combination.

**2.7. Instruments:** The materials of our study included solutions of hematological analyzer of Sysmex – 500 (Germany), reagent of hematological analyzer (Diluent CELLPACK), WBC lyse reagent, Cell clean detergent, tube with EDTA, microscope (Olympus, Japan), slides, cover slide, ethanol 70 %, sterile syringes, tourniquet, and Gaemsa's stain.

### 2.8. Validation of Hematological Analyzer Method

Calibration was performed to compensate for any inaccuracies of the pneumatic, hydraulic, and electric systems which will affect analysis results. This is very important in maintaining the system accuracy, precision and linearity. For calibration, five samples of fresh normal blood were used which meets the following condition: 1) blood of a healthy person who was not taking any medicines, 2) added with EDTA anticoagulant, 3) per – sample whole blood volume to exceed 2 mL. Reference values of calibration was used as the following: five normal blood samples were prepared for calibration of WBC and differential count and were accurately analyzed three times each in accordance with the reference method. The samples were gently mixed sufficiently and analyzed in the whole blood mode.<sup>[8]</sup>

### 2.9. Diphtheria diagnosis

National protocol was be used for confirmation of diphtheria infection in the swab sample of 20 patients.<sup>[9]</sup>  
*Dorset egg medium:* *C. diphtheriae* grows rapidly on these media, producing significant growth in 4–6 hours. The characteristic morphological features of *C. diphtheriae*, especially granule formation, are well developed. In Albert stained smears, particularly from Loeffler serum or Dorset egg cultures, *C. diphtheriae* often appears beaded due to the presence of darkstaining granules in the rods. These granules, known as volutin or metachromatic granules, are energy-storing inorganic polyphosphate units. In some strains the granules form at the ends of the rods. In toluidine blue stained smears, the organisms stain pale blue and the granules dark red-purple. *Tellurite blood agar:* This medium was used as a primary medium for isolating *C. diphtheria* from throat and nasopharyngeal swabs. The isolates were confirmed by biochemical tests. The samples were diagnosed pre and post treatment to monitor the effectiveness of benzylpenicillin.

### 2.10. Diphtheria treatment

As soon as the diagnosis of diphtheria was made clinically, Patients received intravenous benzylpenicillin (Manufactured by Fabrique, Chain ) namely 50,000 U/kg /daily for 10. Patients received an intravenous injection of 20,000 –100,000 IU (Manufactured by Premium, India ) that was diluted in 500 ml normal saline of diphtheria antitoxin in accordance with WHO guidelines.<sup>[3,10]</sup>

### 2.11. Assay of Hematological Parameters

The procedure a the follow : a) Collection and preparing samples: A specified 3 ml amount of venous sample, corresponding to 3 ml of EDTA anticoagulant, was collected. b) Selecting whole blood mode: When the line on the side of the system status area on the LCD screen, the pre – diluted (PD) mode was in use for analysis. c) Inputting sample number: The sample number was set by the incremented value for each analysis and input the sample number when changing. d) Analyzing samples: The sample was mixed sufficiently, remove the plug while taking care not to allow blood scatter, the tube was set to the sample probe, and in that condition, start switch. After collecting the blood samples, they were immediately examined by using Sysmex hematological

analyzer for assaying of WBC, On the other hand, differential count namely neutrophils, basophils, eosinophils, monocytes and lymphocytes were reported by using automatic and manual methods because the assurance the result of hematological analyzer.<sup>[8]</sup>

### 2.12. Statistical Data Analysis

The differences between the females and males groups were analysed by using Excel 2010 and Statistical Process Social Sciences (SPSS) version 15 to calculate the descriptive analysis and paired t - test at  $\alpha = 0.05$  that were used to explore the effectiveness of crystal penicillin pre and post treatment in Hodeidah city, Yemen.

## 3. RESULTS

### 3.1 Characteristics of patients

The background information of personal data on the 90 samples in the children group in Hodeidah city, Yemen was summarized in Table 1. The personal data namely age, and sex, and vaccination status, and clinical history were recorded and the results showed that the age of both males and females included in this study between 1 to 50. The vaccination status 51.11 % vaccinated and 48.88 % unvaccinated.

**Table 1. Demographic data.**

Parameters	Male (n= 38)	Female (n = 52)
Age (Year)	1 – 50	1 – 50
Vaccinated	19	27
Unvaccinated	19	25

### 3.2. Validation of haematological analyzer method

**3.2.1. Linearity:** Linearity was performed for WBC count, and the coefficient correlation ( $R^2$ ) value was more than 0.9995 as a good linear method for all parameters (Table 2).

**Table 2. Linearity of hematological analyzer to assay of WBC and differential count for monitoring the efficacy of antibiotics**

Parameters	Linearity	
	Range	$R^2$
WBC ( $\times 10^9/L$ )	0 - 100	> 0.995

**3.2.2. Precision:** The percent of relative standard deviation (RSD %) for repeatability of the blood indices namely WBC count was presented in Table 3. The maximum RSD was 5 % with WBC, and the minimum RSD was 50 % with basophils.

**3.2.3. Accuracy:** The accuracy (represented by recovery) of hematological analyzer method was determined at the target concentration levels used to construct the hematological profile in Table 3. All recoveries are within acceptable limits ( $\pm 3$ ) for WBC, neutrophils, and lymphocytes ; ( $\pm 2$ ) for monocytes, and ( $\pm 1$ ) for basophils and eosinophils, indicating that the method was suited for the analysis of WBC and differential

count. On the other mean, the accuracy of validated method exhibited well recovery value of (97 – 103 %).

**Table 3. Precision and accuracy of hematological analyzer to assay of WBC and differential count for monitoring the efficacy of antibiotics**

Parameters	Precision (RSD %)		Accuracy (%)
	Repeatability	Reproducibility	
WBC ( $\times 10^9/L$ )	< 5.0	< 5.0	$\pm 3$
Neutrophils (%)	< 16.0	< 16.0	$\pm 3$
Eosinophils (%)	< 40.0	< 40.0	$\pm 1$
Basophils (%)	< 50.0	< 50.0	$\pm 1$
Lymphocytes (%)	< 16.0	< 16.0	$\pm 3$
Monocytes (%)	< 40.0	< 40.0	$\pm 2$

### 3.3. Monitoring efficacy of benzylpenicillin

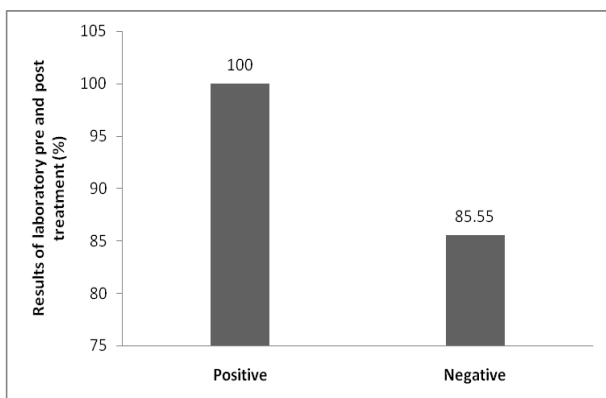
The results showed that the age of the patients included in this study between 1- 50 years with 1.5 : 2.0 male: female. 90 patients were admitted in diphtheria isolation department of CTMID. WBC and differential count were monitored. 77/90 cases ( 85.5 % ) were recovered with benzylpenicillin and DAT, the average of WBC pre - treatment was  $17.9 \pm 4$  ( $\times 10^9/L$ ) and reduced to treatment  $9.4 \pm 2$  ( $\times 10^9/L$ ) post - treatment with significantly statistically different ( $p < 0.05$ ) (Table 4).

WBC at follow up was  $6.68 \pm 1.62$  ( $\times 10^9/L$ ). 13/90 cases (14.44 %) were not response to benzylpenicillin with elevated the WBC and neutrophils, on the other mean, the cases were developed into complication phase to use other treatment guideline. The WBC count, which had reached a peak of 38,200 ( $\times 10^9/L$ ) as maximum value, gradually fell to normal rang post treatment Also, lymphocytes increased gradually with diphtheria infection.

**Table 4: Monitoring the level of WBC and differential count pre and post treatment (mean  $\pm$  standard deviation).**

Parameters	Pre treatment 1 <sup>st</sup> week	Post treatment 2 <sup>nd</sup> week	Follow up after recovery 3 <sup>rd</sup> week
WBC ( $\times 10^9/L$ )	$17.9 \pm 4$	$9.4 \pm 2$	$6.68 \pm 1.62$
Neutrophils (%)	$80 \pm 10$	$63.66 \pm 5$	$54.84 \pm 5$
Eosinophils (%)	$2.7 \pm 1.5$	$2.7 \pm 1.5$	$2.5 \pm 1.4$
Basophils (%)	$0.1 \pm 0.2$	$0.2 \pm 0.2$	$0.1 \pm 0.2$
Lymphocytes (%)	$21.5 \pm 9.5$	$37.5 \pm 6.5$	$51.5 \pm 8.8$
Monocytes (%)	$3.5 \pm 2.1$	$4.1 \pm 2.2$	$5.0 \pm 2.0$

Also, the efficacy of benzylpenicillin was monitored based on microbiological assay. In pre-treatment phase where all cases were positive namely 90 cases (100 %). In post - treatment was recorded 77/90 (85.55 %) negative (Figure 1).

**Figure. 1: Monitoring efficacy of benzylpenicillin pre and post treatment based on microbiological assay.**

## 4. DISCUSSION

Clinicians namely pediatric, tropical medicine, and clinical pharmacologist are frequently asked to monitor the effects of benzyl penicillin treatment with the objective of ensuring safe and effective therapy. Already in monitoring of antibiotic includes drug concentration to attain therapeutic response without dose related toxicity, or to confirm compliance.<sup>[1,2]</sup> But our study monitored

the efficacy of benzylpenicillin based hematological parameters namely WBC for a response to treatment and microbiological parameters (antimicrobial effectiveness). Also the clinical response to treatment was used. However, the benzylpenicillin acts on inhibition of cell wall synthesis of bacteria and is the drug of choice for streptococcal, pneumococcal, gonococcal and meningococcal infections, and is also useful for treatment of anthrax, diphtheria, gas gangrene, leptospirosis, syphilis, tetanus, yaws and Lyme disease in children.<sup>[11]</sup>

Findings of the present study showed that almost all the WBCs of diphtheria patients sampled from Hodeidah, Yemen (collected systemically from diphtheria patients) were found to be more than normal values. These results were estimated according to direct hematological analyzer that was validated using a classical approach for the assay of WBC and related parameters. This approach gives enough guarantees for the results that will be generated by this method during blood analysis.<sup>[12,13]</sup> Calibration Verification Assessment (CVA) is a multi-level product designed to verify the reportable range for WBC, and other cells count. Linearity verifies the



reportable range of the WBC. On the other mean, the lab can simultaneously verify both the analytical measurement interval/range and calibration verification for WBCs.<sup>[14]</sup> On the other hand, demonstrate that it can obtain performance specifications comparable to those established by the manufacturer for the following performance characteristics: namely accuracy and precision.

Monitoring efficacy of benzylpenicillin and response to treatment of an infection can be assessed using both clinical and microbiological parameters.<sup>[15,16]</sup> Clinical parameters of improvement include symptoms and signs (eg, a decrease in fever, tachycardia, good swallowing), laboratory values (eg, decreasing leukocyte count within normal rang). Although hematological criteria namely WBC and neutrophils are commonly used as economical indicators in assessing response to infectious disease therapy in Hodeidah outbreak management, WBC improvement can frequently lag behind clinical improvement, and routine follow-up of diphtheria infection is always necessary.

Increase of lymphocytes gradually were observed in our study due to the lymphocytes which make antibodies that can bind to pathogen (toxin), block pathogen (toxin) invasion, activate the complement system, and enhance pathogen (toxin) destruction. Also, increase of neutrophils were reported in all diphtheria patients because the neutrophils are active in phagocytosing bacteria and the most common cell type seen in the early stages of acute inflammation. They make up 40-75 % of total leukocyte count in human blood and lymphocytes make up 20 – 45 %.<sup>[17-19]</sup>

## 5. CONCLUSION

In conclusion, monitoring diphtheria patients with antibiotic treatment is important to ensure successful treatment, identify adherence problems and determine whether benzylpenicillin regimens should be switched in case of treatment failure. In the absence of better criteria namely monitoring of concentration in blood to predict treatment failure, it is important to use WBC count and clinical assessment to identify those at the highest risk of disease progression and mortality. On the other hand, the monitoring provides an early and more accurate indication of treatment failure and the need to switch from first-line to second-line drugs, reducing the accumulation of drug resistance mutations and improving clinical outcomes.

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