Original Research Article

A STUDY ON PROCALCITONIN LEVELS IN PATIENTS HAVING SEPSIS WITH A SPECIAL EMPHASIS ON SEPSIS DUE TO TROPICAL INFECTIONS:A CROSS-SECTIONAL STUDY

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ABSTRACT

Background/Aims: To study the levels of Procalcitonin in patients presenting with sepsis, especially those with tropical sepsis at a rural population catering teaching hospital.

Study Design: Cross-sectional study.

Place and Duration of study:This cross-sectional study was carried out at the Microbiology and General Medicine Department of SBKS Medical Institute & Research Centre affiliated to Sumandeep Vidyapeeth Deemed to be University.After obtaining necessary approval from the ethics committee this study was conducted between September 2012 and December 2015.

Methodology: With the hypothesis that different microbiological agents, especially tropical infections, will evoke diverse host responses in sepsis, this study was undertaken.It was aimed at determining the levels of Procalcitonin in patients presenting with sepsis along with those having tropical sepsis. Patients with age >18 years and diagnosed clinically as sepsis by 1992/2001 definition were included in the study. PCT levels were determined in a total of 155 patients. It was carried out by a semi-quantitative test till March 2015 and then later using a QDx Instacheck quantitative test kit and QDx Instacheck Reader. Ninety eight with tropical sepsis, 46 with non-tropical and 11 with sepsis due to unidentified etiologies were tested making a total of 155 patients.

Results: Of the total 155 patients tested, 43 had values of ≤0.5ng/ml, 32 had values of >0.5 to <2 ng/ml, 55 had ≥2 to <10ng/ml and 25 had values of ≥10 ng/ml. Thus a larger number of patients showed the values ranging between 2ng/ml and 10ng/ml.

Conclusions: Overall PCT was found raised in mainly bacterial, fungal and malarial infections especially with the values of ≥2 to <10 ng/ml whereas those with dengue had values of ≤0.5ng/ml. Thus PCT values were not much useful in differentiating infections due to bacteria, fungi or malaria but those with dengue had values ≤0.5ng/ml. Values of >10ng/ml were mainly associated with severity and mortality.

Keywords: Procalcitonin , Sepsis , Tropical sepsis , Malaria , Dengue.

ABBREVIATIONS

PCT : Procalcitonin
SIRS : Systemic Inflammatory Response Syndrome
DAMA : Discharge Against Medical Advice

1. INTRODUCTION

Infectious illnesses plagues all age groups, particularly affecting those living in tropical areas[1].Tropical diseases are defined as diseases that are prevalent in, or unique to tropical and subtropical regions[2]. These diseases are relatively less prevalent in temperate climates, due to the occurrence of a cold climate, which controls the insect population by forcing them to hibernate [3].In the tropics, warm climate, poverty, lack of education and poor sanitation provide an ideal environment for pathogens, vectors and intermediate hosts to flourish [4].

Though the new definition of sepsis has come , earlier sepsis was defined as a clinical syndrome characterized by a severe infection and systemic inflammatory response syndrome (SIRS; i.e., a serious inflammatory reaction that is diagnosed when two or more of the following criteria are present: temperature > 100.4 ºF/38 ºC or < 96.8 ºF/36 ºC; heart rate > 90/min; respiratory rate > 20/min or PaCO2 < 32 mm Hg; and WBC count > 12,000/mm3, < 4,000 mm3, or > 10% immature forms). SIRS can occur with or without an infection, but sepsis can only be diagnosed when SIRS occurs in the presence of a suspected or confirmed infection (i.e. Sepsis = SIRS + Infection) . Severe sepsis (i.e., sepsis with multiple-organ dysfunction – hypoperfusion or hypotension) can lead to septic shock (i.e., severe sepsis with arterial hypotension despite adequate fluid resuscitation) and death.[5].This definition is considered for the present study.

Early diagnosis with prompt antimicrobial therapy is crucial in the treatment of sepsis and reducing mortality[6].

Procalcitonin (PCT) has emerged as a promising marker for the diagnosis of systemic infections[7,8].PCT is a member of the calcitonin (CT) superfamily of peptides. It is a peptide consisting of 116 amino acid with an approximate molecular weight of 14.5 kDa, and its structure can be divided into three parts : amino terminus (represented by the ball and stick model), immature calcitonin, and calcitonin carboxyl-terminus peptide 1.[9] Under normal physiological conditions, active CT is produced and secreted in the C-cells of the thyroid gland after proteolytic cleavage of PCT, meaning, in a healthy individual, PCT levels in circulation are very low (<.05 ng/mL).[10]

Procalcitonin (PCT) is synthesized by a large number of tissues and organs in the body as a response to invasion by pathogenic bacteria, fungi, and some parasites[11].Microbial infections induce a universal increase in the CALC-1 gene expression and a release of PCT (>1 µg/mL)[10]. Expression of this hormone occurs in a site specific manner[9] .In healthy and non-infected individuals, transcription of PCT only occurs in neuroendocrine tissue, except for the C cells in the thyroid. The formed PCT then undergoes post-translational modifications, resulting in the production small peptides and mature CT by removal of the C-terminal glycine from the immature CT by peptidylglycine α-amidating monooxygenase (PAM).[12] In a microbial infected individual, non-neuroendocrine tissue also secretes PCT by expression of CALC-1. A microbial infection induces a substantial increase in the expression of CALC-1, leading to the production of PCT in all differentiated cell types.[13] The function of PCT synthesized in non-neuroendocrine tissue due to a microbial infection is currently

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unknown, but, its detection aids in the differentiation of inflammatory processes[9].Thus, in this study we have evaluated the levels of Procalcitonin in patients having sepsis with a special emphasis on patients having tropical sepsis.

2. METHODOLOGY

This cross-sectional study was carried out at the Microbiology and the General Medicine Department of SBKS Medical Institute & Research Centre affiliated to Sumandeep Vidyapeeth Deemed to be University. For the measurement of PCT , a blood sample (2ml clot) was collected in a sterile manner. The samples were immediately transported for the Procalcitonin test.

2.1 Objectives of the study

To compare and evaluate PCT levels in various tropical and non-tropical causes of sepsis.
Determine the utility of PCT in early detection of sepsis.

2.2 Inclusion Criteria

The inclusion criteria for the patients were as below:

1. Adult patients with age >18 years with

Two or more of the following conditions along with a proven or suspected microbial aetiology:

1. Fever (oral temperature >38°C) or hypothermia (<36°C);
2. Tachypnea (>24 breaths/min);
3. Tachycardia (heart rate >90 beats/min);
4. Leukocytosis (>12,000/L), leukopenia (<4,000/L), or >10% bands

2.3 Exclusion Criteria

The exclusion criteria for the patients were as below:

1. Patients with age <18 years
2. Patients NOT having 2 or more signs of the following: tachycardia, tachypnea, leukocytosis or fever/hypothermia

2.4 Procalcitonin Test:

2.4.1 Principle:

Detection & measurement of PCT from serum samples was carried out using PCT-Q kit (CPC Diagnostic Pvt. Ltd.) It is a semi-quantitative test based on immunochromatographic method. The test uses a monoclonal mouse anti-catacalcin antibody conjugated colloidal gold (tracer) and a polyclonal sheep anti-calcitonin antibody (solid phase). After the test sample has been applied to test strip, the tracer binds to the PCT in the sample and a marked antigen-antibody complex forms. This complex moves by capillary action through the test system and, in the process passes through the area containing the test band. Here, the marked antigen-antibody complex binds to the fixed anti-calcitonin antibodies and forms a sandwich complex.
Procedure: The test was performed according to the manual as follows:

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First, a 200 µl of the patient's serum sample was added, using the dropper provided in the kit, into the cavity of the card & the card was kept for 30 minutes. After 30 minutes the results were read. A coloured band is formed at "C" region and also at "T" region. The intensity of the colour of the band at "T" region is compared with colour on the reference card. The colour corresponds to the value of PCT concentrations.
Alternative Method for PCT: (March 2015-December 2015):
Since March 2015, the PCT was determined using the QDx Instacheck PCT Kit and QDx Instacheck Reader.
Principle:
QDx Instacheck Reader is a fluorescence scanning instrument for antigen-antibody reactions based on fluorescence technology. The fluorescent light is collected together with the scattered laser light. Intensity of the fluorescence is scanned and converted to electric signal which is proportional to the intensity of fluorescence produced.
Procedure:
A 150µl of serum sample was added to the well in the cartridge containing detection buffer. The lid of the tube was then closed tightly and the mixture was mixed properly by shaking the tube 10 times.
Then 75 µl of sample mixture was taken and added to sample well in the cartridge.
The sample-loaded-cartridge was placed in the cartridge holder in the QDx Instacheck Reader and the "Select" button was pressed on the reader after which the reader starts scanning the test cartridge.
The result was displayed on the screen of the reader i.e. PCT concentration in the test serum sample in terms of ng/mL.

2.4.2 Interpretation:

The levels PCT were interpreted as follows

Table:1-Interpretation of PCT concentration. Adapted from Meisner M.[14]

Concentration	Interpretation	Diagnostic utility
≤ 0.05 ng/ml	Normal value	
0.05-0.5 ng/ml	Local infection could be possible	Local infection without systemic signs-it may be associated with low PCT levels. Also if PCT is done during early phase <6 hrs; needs to be reassessed again after 6-24 hours Low risk of progression to severe systemic infection (severe sepsis)
≥0.5 and < 2.0 ng/ml	Systemic infections/ Sepsis could be possible	Indicates a moderate risk for progression to severe systemic infection (severe sepsis) The patient should be closely monitored both clinically and by re-assessing PCT within 6-24 hours
≥2.0 and < 10 ng/ml	Systemic infections/ Sepsis could be possible	Indicates a high risk for progression to severe systemic infection (severe sepsis)

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≥10.0ng/ml	Systemic Inflammatory response syndrome due to bacterial sepsis or Septic Shock could be possible	Indicates a high likelihood of severe sepsis or septic shock

3. Results and Discussion:

PCT levels were determined in a total of 155 patients. It was carried out by a semi-quantitative test till March 2015 and then later using a QDx Instacheck quantitative test kit and QDx Instacheck Reader. Ninety eight with tropical sepsis, 46 with non-tropical and 11 with sepsis due to unidentified etiologies were tested making a total of 155 patients.

Table:2- Distribution of cases according to various etiologies.

Sr no.	Type	Number of patients
1	Tropical sepsis	98
2	Non-tropical sepsis	46
3	Unidentified etiology	11
Total		155

Of the total 155 patients tested, 43 had values of ≤0.5ng/ml, 32 had values of >0.5 to <2 ng/ml, 55 had ≥2 to <10ng/ml and 25 had values of ≥10 ng/ml. Thus a larger number of patients showed the values ranging between 2ng/ml and 10ng/ml.

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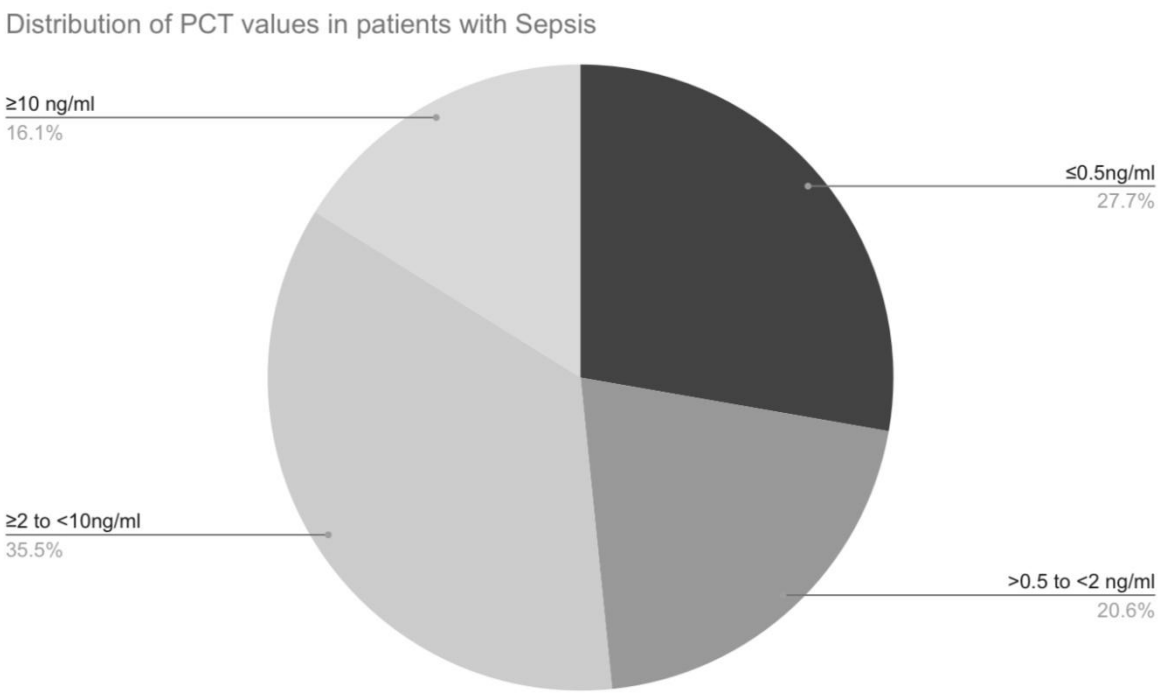

Figure 1: Distribution of PCT values in patients with sepsis (n=155)

Of those 55 patients having PCT value ≥2 to <10ng/ml, 1 was HBV positive , 2 were HAV positive , 6 had dengue , 22 had malaria , 21 had only bacterial isolates while 3 had bacterial+fungal isolates together.

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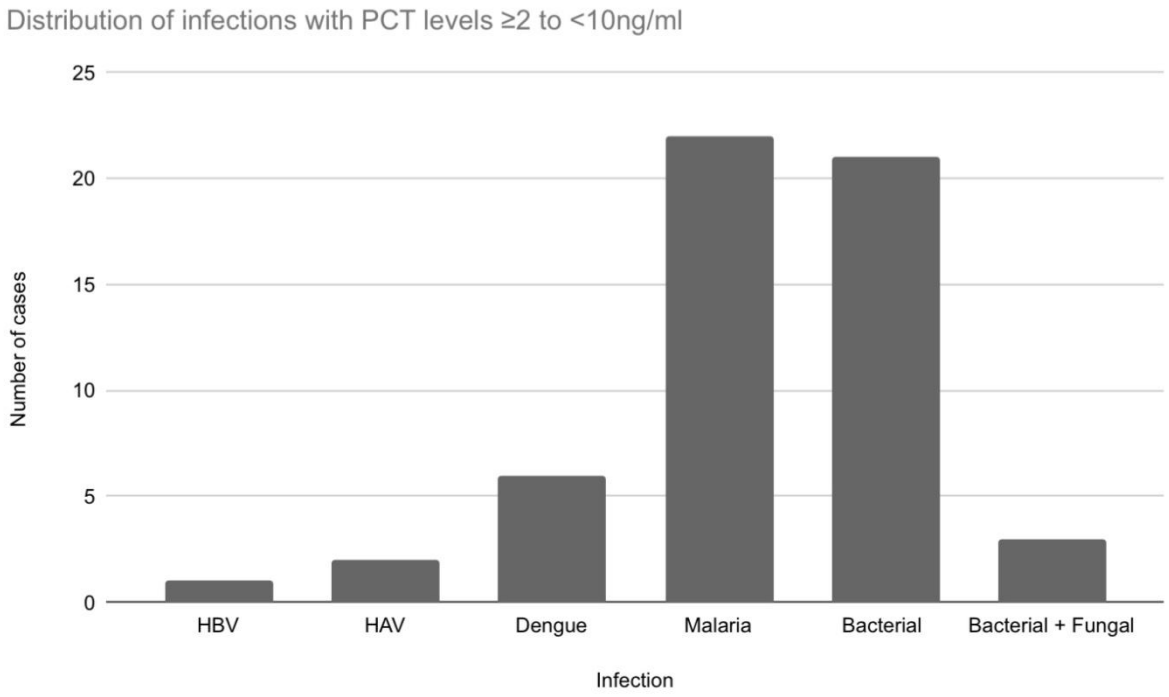

Figure 2: Distribution of infections with PCT levels ≥2 to <10ng/ml (n=55)

Of the 155 patients tested for PCT, 57 belonged to the Non-Survivors group. Thirteen of the Non-survivors had PCT values ≤0.5, whereas 11 had values >0.5 to <2 and 23 had values ≥2 to <10 and 10 patients had values ≥10ng/ml. Amongst those with values ≥10ng/ml, 8 had tropical sepsis and 2 had non-tropical sepsis.

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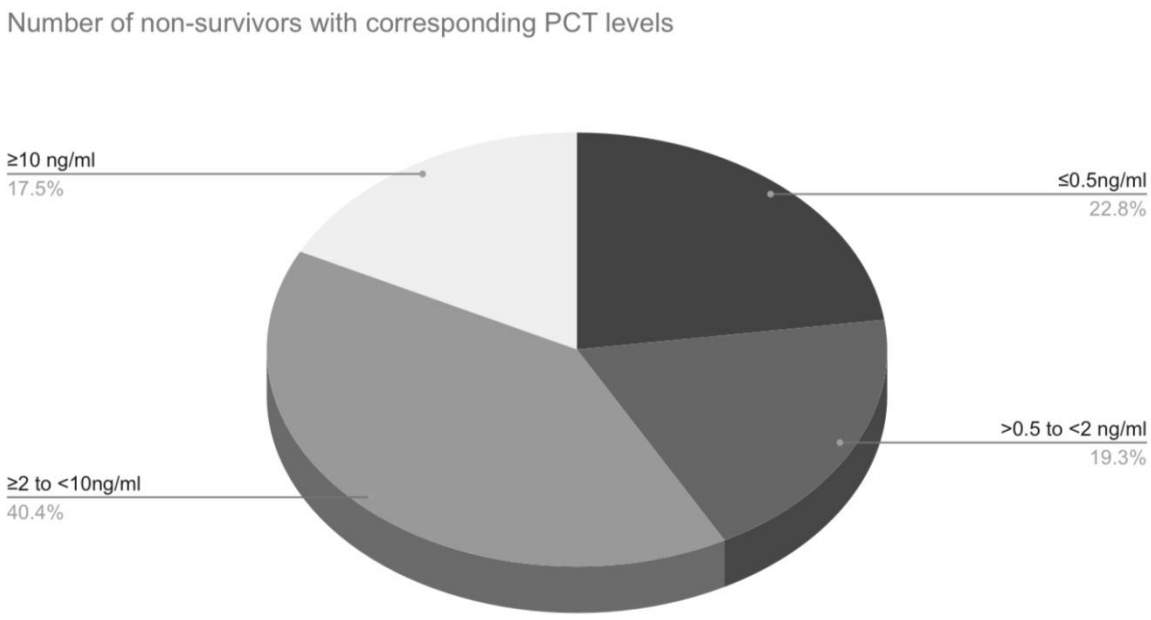

Figure 3 : Number of non-survivors with corresponding PCT levels (n=57)

Of the total 155 tested, 25 had values of PCT ≥ 10ng/ml. The outcome amongst these patients is as shown below in the chart. Accordingly 11 survived, 10 did not survive and for 4 patients the outcome was not known.

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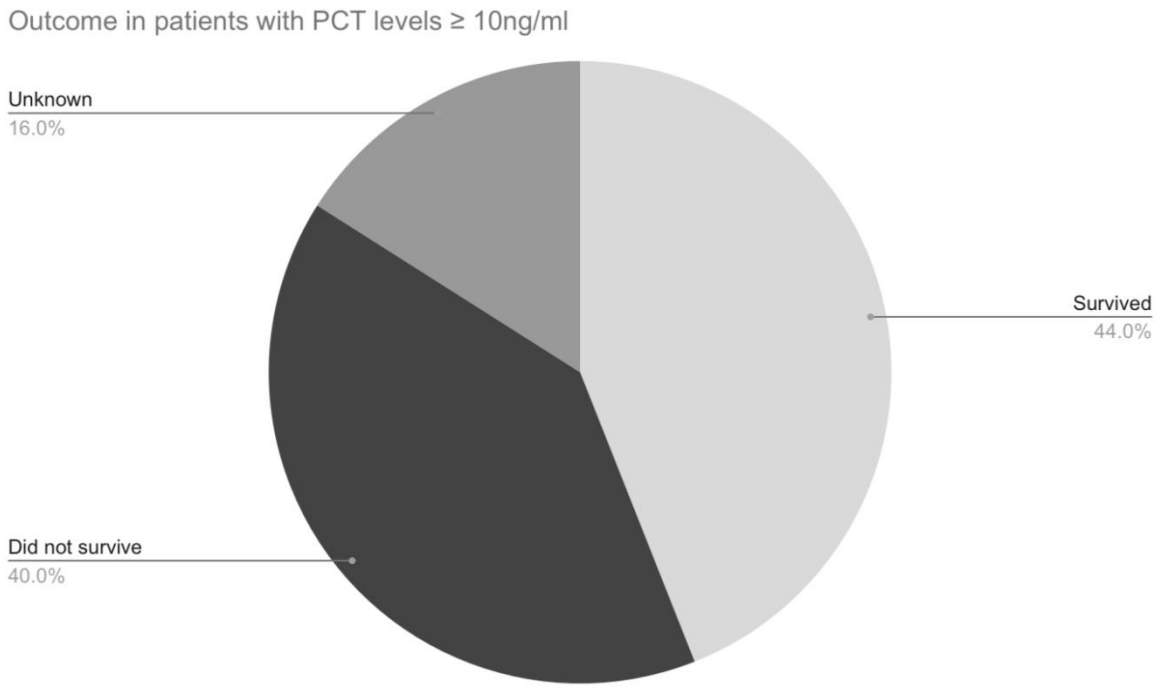

Figure 4: Outcome in patients with PCT levels ≥ 10ng/ml

PCT values amongst different groups of microbial etiology i.e. due to malaria, dengue and only bacterial infection were analyzed. These are shown in the following charts. As shown in the chart amongst the 54 malaria patients tested for PCT, the maximum number of patients i.e. 22 showed PCT values of ≥2ng/ml to <10ng/ml, followed by 16 patients with values of >0.5ng/ml to <2ng/ml and 10 patients with ≥10ng/ml and least i.e. 6 patients with ≤0.5ng/ml.

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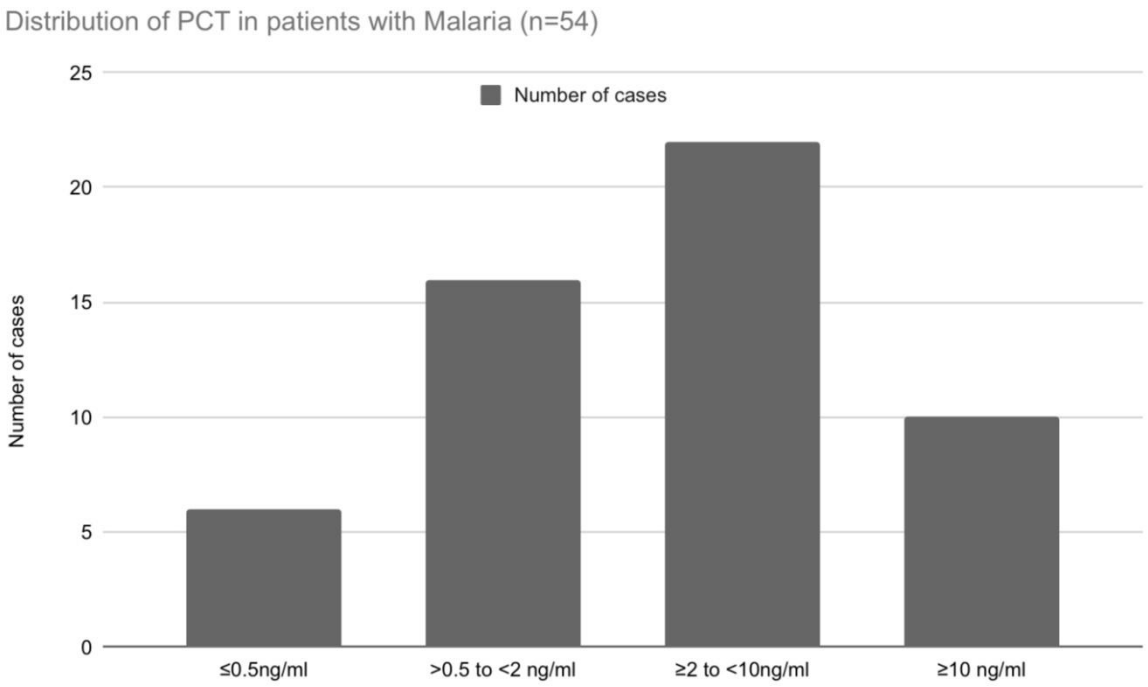

Figure 5: Distribution of PCT in patients with Malaria (n=54)

Amongst those patients with only bacterial infections 45 were tested for PCT. Of these, 44.44% had values of ≥2 to <10 ng/ml followed by 22.22% with ≥10 ng/ml.

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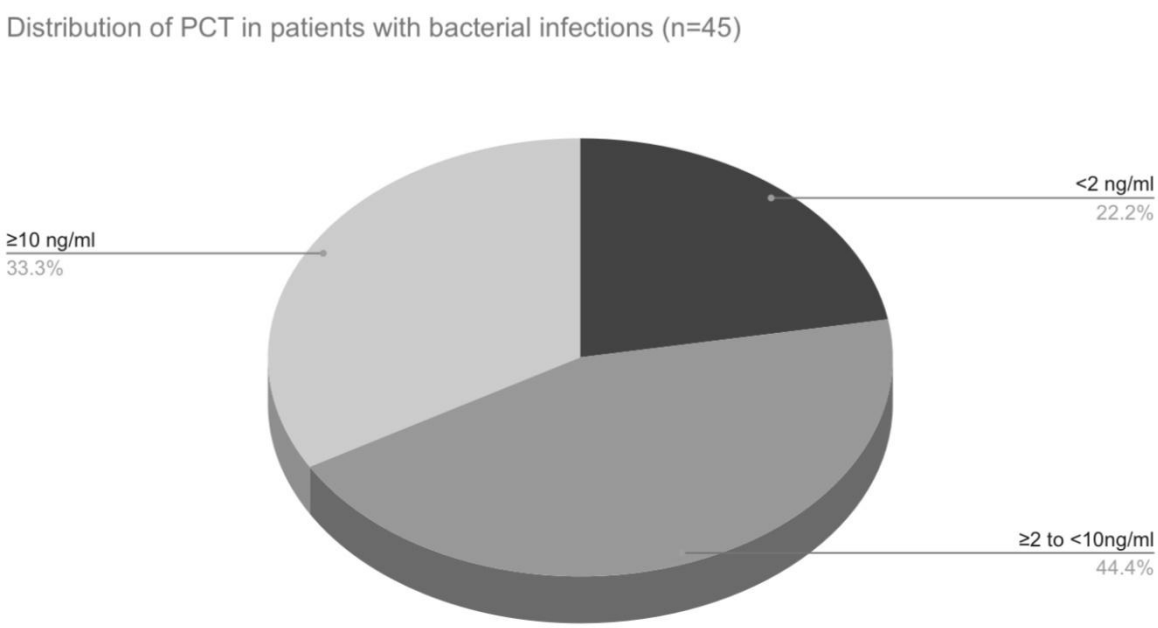

Figure 6 : Distribution of PCT in patients with bacterial infections (n=45)

Amongst the 32 dengue patients tested with PCT, majority i.e. 62.5% had values of ≤0.5ng/ml followed by 18.75% with values ≥2<10 ng/ml.It was also noted that patients having dengue with shock had PCT levels >10 ng/ml.

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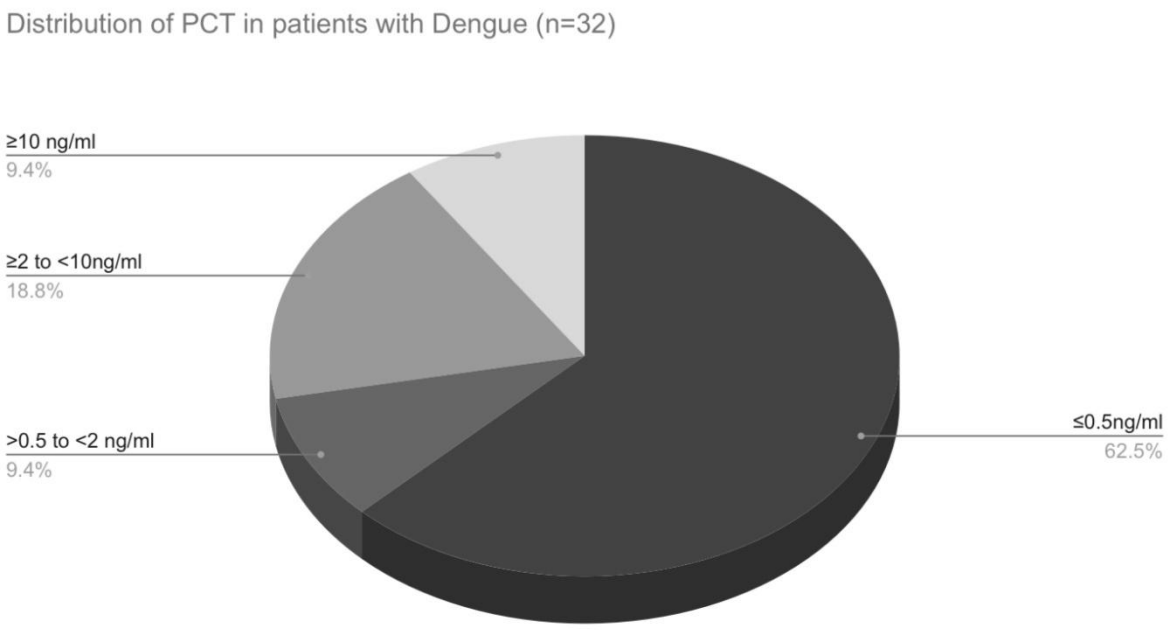

Figure 7: Distribution of PCT in patients with Dengue (n=32)

Procalcitonin can be used as a prognostic marker in the patients of sepsis . A study done by Lakhani Som J *et al* on sepsis due to skin and soft tissue infections , moderate to high increase in PCT was found in patients who died.[15]

4. CONCLUSION

Overall PCT was found raised in mainly bacterial, fungal and malarial infections especially with the values of ≥2 to <10 ng/ml whereas those with dengue had values of ≤0.5ng/ml. Thus PCT values were not much useful in differentiating infections due to bacteria, fungi or malaria but those with dengue had values ≤0.5ng/ml. Values of >10ng/ml were mainly associated with severity and mortality. Similar findings have been reported by Gaini S et al (II) who suggested that PCT should not be introduced as a routine test in diagnosing infection and sepsis in patients with sepsis or community acquired mild infections. PCT is mainly a marker of bacteremia and severity.

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CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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