Comparative Study between Neopterin and Alvarado Score in the Diagnosis of Acute Appendicitis and Its Severity

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Abstract

BACKGROUND: Acute appendicitis (AA) remains a complex case even for experienced surgeons. Rate of negative appendectomy is 5–40% and delayed intervention result in perforated appendicitis in 5–30% of cases.

AIM: The aim of the study was to evaluate NPT as a marker for the diagnosis of AA concerning its severity. And compare the diagnostic value of it with the ALV scoring system.

METHODS: One hundred twenty patients presented with signs and symptoms of AA and underwent appendectomy, only 84 patients proved to be AA by histopathological examination, were included in the study. Blood samples for neopterin (NPT) estimation and Alvarado (ALV) score was calculated. Control group consists of 45 healthy individual.

RESULTS: NPT levels were significantly higher in patients' group than control with p = 0.001 at a cutoff point 5.3 nmol/L. The diagnostic accuracy of NPT was higher than ALV score. NPT sensitivity, specificity, positive predictive value, and negative predictive value were 85.4%, 76.9%, 89%, and 70%, respectively.

CONCLUSION: NPT significantly elevated in patient with AA and has a high diagnostic accuracy, with correlation to clinical features and severity of the inflammation.

Introduction

Acute appendicitis (AA) is a common abdominal emergency with a life time prevalence of about 7%. The clinical diagnosis of AA remains a challenge to surgeons. The clinical diagnosis helps in patients who presented with classical signs and symptoms, but a typical presentations may end in diagnostic confusion and delay in management which will increase the possibility of complications, including appendicular mass, appendicular perforation, sepsis, and even death. Symptoms are usually not specific and overlap with other diseases. Despite all improvement in clinical and laboratory diagnosis and the multiple scoring systems to guide the diagnosis, the decision to operate or not remains challenging [1].

AA can affect people at any age, usually between the ages of (10 and 30) years, slightly more common in males, with a male to female ratio 1.4:1. Perforation is found in about 13–20% of patient with AA [2]. Appendicitis is a multi-factorial disease, but faecoliths, foreign body, malignancy, and lymphoid hyperplasia during an infection are possible causes. Negative appendectomy might not only expose the patient to the risk of surgical operation but also increase the risk of myocardial infarction related to surgical removal of appendix and tonsils as it has been reported [3].

Diagnostic approaches include history of present illness, physical examination, laboratory tests, and imaging modalities.

Abdominal pain is the main presenting complain in patients with AA started usually as an umbilical colicky pain then become sharp and constant which migrate to the right iliac fossa. Other symptoms such as loss of appetite, nausea, and vomiting may also present. Less typical symptoms warrant a period of observation and re-evaluation, this approach could result in unnecessary prolong hospital stay and delayed management [2]. On clinical examination, right lower abdominal tenderness on palpation is the most important finding which may or may not be associated with rebound tenderness [3].

Many laboratory investigations used to help in the assessment of a patient with suspected AA, for example, urine analysis, which may reveal another diagnosis such as urinary tract infection or renal stone, blood tests may reveal elevated white blood cell (WBC), elevated C reactive protein (CRP) which makes appendicitis more likely [4].
Biochemically, it derives from guanosine triphosphate, it belongs to the chemical group known as pteridines. It is synthesis from human monocyte-derived macrophage and dendritic cells upon stimulation with pro-inflammatory cytokine interferon-gamma. Increased NPT concentrations are commonly observed with diseases in which the cellular (T-helper 1 [TH1]–type) immune system is involved. These include primarily infections with viruses and intracellular bacteria, auto-immune syndromes, malignancies, and allograft rejection episodes. Measurement of NPT concentrations in body fluids such as blood, serum, spinal fluid, and urine provides information about activation of cellular system in human, under control of TH cells type 1 [7].

High NPT production is associated with increased production of reactive oxygen species. NPT is also used to estimate the extent of oxidative stress elicited by the immune system [7].

NPT could be elevated in infections whether viral (HIV), bacterial (Escherichia coli, Borrelia, and Helicobacter pylori), or parasitic (malaria), auto-immune diseases such as rheumatoid arthritis and systemic lupus erythematosus. Furthermore, in malignant tumor (gynecological and hematological tumors) and allograft rejection episodes. Blood NPT concentrations are age-dependent, being higher in children and elderly people [8], [6].

The objective of the study was to evaluate NPT as a marker for the diagnosis of AA concerning its severity. And compare the diagnostic value of it with the ALV scoring system.

Materials and Methods

This case–control study was conducted in the Emergency Department at Al-Kindy Teaching Hospital between February 2018 and September 2018, where the patients were suspected of having AA presenting with acute abdominal pain of <48 h duration and were operated on after full history taking, clinical examination, and essential laboratory tests were done. Samples from the healthy individuals were collected as a control group.

A pre-check list was filled through direct interview with the patient, the chick list included: Age, gender, NPT level, and ALV score.

The decision of appendectomy was solely based on surgeon’s clinical judgment after taking into consideration all the findings of clinical, laboratory, and radiological investigations.

Patients were monitored following admission, surgery until discharged well form the hospital. The daily follow-up included monitoring of vital signs, general appearance, and abdominal examination.

The patients underwent appendectomy under general anesthesia in the emergency theatres at Al-Kindy Teaching Hospital. The final diagnosis was done by histopathology examination of the resected specimen macroscopically and microscopically. All of the specimen was investigated in the Histopathology Department at Al-Kindy Teaching Hospital. Where mild AA defined as acute mucosal and submucosal inflammation while severe AA defined as suppurative and gangrenous appendicitis.

Inclusion criteria

All patients aged above 16-year-old suffered from signs and symptoms suggesting AA within 48 h duration.

ALV score (Table 1) is a 10-clinical scoring system based on history, physical exam, and laboratory investigation to describe the likelihood of AA. Those with low scores are unlikely to have AA. Score 5–6 is possible for AA, score 7–8 is probable, and score >9 is very probable.

From each patient, an extra 5 ml of blood was collected in the ER. Samples were assayed according...
to the manufacture’s recommended procedure by trained biochemist at Al-Kindy College of Medicine Biochemistry Lab.

This enzyme immunoassay is evaluated for the manual use and especially for the automated use with (Dade Behring Holdings, Inc., Germany) ELISA processor for the determination of NPT in serum.

**Statistical analysis**

The collected data were introduced in the Microsoft excel sheet and located into the IBM-SPSS version 24 statistical package was used in statistical analysis.

Descriptive statistical analysis was presented using mean ± standard deviation (SD), frequency distribution tables, and graphs.

Inferential statistic was displayed using two sample t-test, Chi-squared test, measurement of diagnostic accuracy, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).

Receiver operating characteristic (ROC) curves with calculation of total area under the curve (TAUC) and the significance of the result were measured and coordination of the curves was presented in tables to calculate sensitivity and specificity of the test at different cutoff points.

p < 0.05 was considered as the cutoff point for discrimination of significances.

**Results**

A total of 120 patients had been seen in the emergency department (ED) with signs and symptoms of AA which underwent appendectomies, only 84 patients were proven AA by histopathology examination. The mean age were 23.24 ± 6.74, 58% of patients were male and 42% were female, as shown in Figure 1 (distribution of cases according to gender).

Table 2 and Figure 2 (distribution of cases according to severity) show that 31% of appendicitis were mild and 69% were severe, the mean of the ALV score was 7 ± 1.4, 42.9% of cases got the score (5–6), in the rest of the patients the score was more than 6. The mean NPT level was 12.53 ± 1.57 nmol/L, 34.5% of studied patient scored low NPT level (<5.3 nmol/L).

**Table 2: Distribution of studied cases according to essential studied characteristics**

| Parameter          | Number | Percentage (%) | Mean ± SD  |
|--------------------|--------|----------------|------------|
| Gender             |        |                |            |
| Male               | 49     | 58             |            |
| Female             | 35     | 42             |            |
| Neopterin level    |        |                |            |
| Low                | 29     | 34.5           | 12.53 ± 1.57 |
| High               | 55     | 65.5           |            |
| Alvarado score     |        |                |            |
| 5 and 6            | 36     | 42.9           | 7 ± 1.4    |
| >6                 | 48     | 57.1           |            |
| Severity of appendicitis |    |                |            |
| Mild               | 26     | 31             |            |
| Severe             | 58     | 69             |            |

In Table 3, the mean NPT level among the case group was significantly higher than that of the control group with p = 0.001.

In Table 4, a significant association between high NPT level and severe appendicitis with p = 0.001. Table 5 showed that NPT was more sensitive yet less specific than that in ALV score, and the diagnostic accuracy of NPT was higher than that of the score.

Figures 3 and 4 show the severity of appendicitis in male and female patients, respectively, while Tables 6-8 show the association between NPT and the ALV score, and severity of appendicitis in the same patients.
Discussion

For the past two centuries, AA has been the most common indication for emergent abdominal surgery. The rate of diagnostic errors of AA cases still remains approximately 20–45% despite the widespread use of imaging techniques and clinical scoring systems [9], [10].

A recent study in Turkey by Kose et al. showed that the development and/or use of scoring systems do not significantly improve the diagnostic accuracy of AA [6]. Hence, new studies have been made to evaluate some inflammatory markers as a discrimination factors in patient with AA such as interleukin 6 (IL-6), S100A8/A9, CRP, and NPT [12], [13], [14], [15].

NPT is a low molecular weight aromatic pteridine molecule produced mainly by activated monocytes and macrophages, and it serves as a marker for cellular immune system activation [16]. It is shown that there is an increase in the levels of NPT with sepsis, bacterial and viral infections, and malignancy [6].

Table 4: Association of NPT and ALV score with severity in all cases

| Variables          | Severe | Mild |
|--------------------|--------|------|
| NPT                | Count  | Count |
| High               | 49     | 6    |
| Low                | 9      | 21   |
| ALV                |        |      |
| High               | 43     | 5    |
| Low                | 15     | 21   |
| p-value            | 0.001  | 0.001 |

Table 5: Comparison between NPT and ALV score sensitivity, specificity, PPV, NPV, and accuracy

| Parameter          | NPT (%) | ALV (%) |
|--------------------|---------|---------|
| Sensitivity        | 85.4    | 74.1    |
| Specificity        | 76.9    | 80.8    |
| PPV                | 89      | 89.6    |
| NPV                | 70      | 58.3    |
| Accuracy           | 82.1    | 76.2    |

Table 6: NPT and ALV score presented as total area under the curve (all studied cases)

| Test result variable(s) | Area | Std. Error | p-value | 95% confidence interval (CI) |
|-------------------------|------|------------|---------|-------------------------------|
| NPT                     | 0.862| 0.041      | 0.001   | (0.763–0.941)                |
| ALV                     | 0.890| 0.039      | 0.001   | (0.793–0.937)                |

NPT: Neopterin; ALV: Alvarado.

In this study, we analyzed 84 patients presented with signs and symptoms of AA. Their mean age was 23.24 ± 6.74, and they were 35 females and 49 males. We evaluate the diagnostic value of NPT at a cutoff value 5.3 nmol/L and ALV score ≥7.

Table 7: Association between NPT level, ALV score, and severity of appendicitis in male cases

| Variables          | Severe | Mild |
|--------------------|--------|------|
| NPT                | Count  | Count |
| High               | 19     | 2    |
| Low                | 4      | 10   |
| ALV                |        |      |
| High               | 20     | 3    |
| Low                | 6      | 7    |
| Sensitivity=87%, specificity=75%, PPV=86%, NPV=75%, accuracy=82.8%, NPT: Neopterin; ALV: Alvarado. |

We found significant differences in the plasma concentration of NPT in patients with AA compared to control group. NPT diagnostic accuracy was higher than ALV score (82.1% and 76.1%, respectively). Both the NPT and ALV score were correlated with the severity of AA with a p = 0.001 for each.

Clinical usefulness depends mainly on cutoff points that most accurately discriminate between patients with AA and those without. To find this cutoff point, ROC curves were calculated and the ideal cutoff point was assessed as the maximum sum of sensitivity and specificity of the marker. The overall accuracy of the marker was presented by the area under the curve (AUC).
In our study, the AUC of NPT was found to be slightly higher than that of ALV score, while the CI were 0.783–0.941 and 0.783–0.937 for NPT and ALV score, respectively, for all cases. In male patients group, NPT was more sensitive with higher diagnostic accuracy than ALV score with 81.6%, 65.7%, and 71.4%, respectively. In female patients' group, ALV score was more sensitive than NPT with 87% and 82.6%, respectively, while the diagnostic accuracy was the same (82.8%).

NPT was found to be more sensitive in male patient group than female patient group, while females patient group had a higher PPV. Compared to ALV score, NPT was more sensitive in male patients group, while in female patient group, NPT was less sensitive.

A similar study had been conducted in Turkey by Dal, where 100 patients studied for serum CRP, pro-calcitonin, and NPT and which were significantly higher in patients with focal appendicitis. They concluded that these markers diagnostic value and should be correlated with clinical features [18].

Another study in Turkey about the role of NPT levels in the diagnosis of AA but on rabbits which suggest that NPT could be used as marker in the diagnosis of AA. The sensitivity, specificity, PPV, and NPV were 87.5%, 100%, 100%, and 78.5, respectively [19].

In a patient with clinically atypical presentation of AA and non-specific signs and symptoms, usually kept for observation and re-evaluation which may result in unnecessary prolong hospital stay and might be a delayed definitive treatment. This study shows that NPT can help to identify people who are more likely to have AA and can decrease the need for unnecessary hospital admissions and to consider an early appendectomy in cases with significantly high NPT level to decrease postoperative complication.

Conclusion

NPT was found to be a sensitive, non-specific test with high PPV and high diagnostic accuracy. Hence, it can decrease the false positive patients, the admissions for observation, and the unneeded appendectomies.

NPT has a good correlation with the severity of the inflammation of the appendix. Higher levels of NPT indicate a priority for surgical intervention before complications develop and may offer better anticipation for the difficulty of the operation and a possible complication.

Although NPT had a higher sensitivity in the male patient group than female patient group, the diagnostic accuracy was the same.

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Ethical consideration

The proposal of the study was fully discussed and approved by the researcher and the ethical committee of Arabic board of surgery before the researcher starts collecting data. The agreement of health authority of Al-Kindy Teaching Hospital was approved before starting the study.

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