

Procalcitonin as the Biomarker of Inflammation in Diagnosis of Appendicitis in Pediatric Patients and Prevention of Unnecessary Appendectomies

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Abstract Numerous diseases mimic appendicitis, and it is often difficult to rule it out on the basis of clinical presentation. Concentration of procalcitonin selectively increases in inflammatory conditions and determination of its level can help in the diagnosis of acute appendicitis. A prospective, single centre based observational study carried out at our tertiary care institute. Twenty eight patients were admitted with preliminary diagnosis of acute appendicitis. The control group involved around 12 healthy children. Serum Procalcitonin concentration was measured in all patients using the ‘Immunoluminometric Method’ (LUMI-Test PCT), besides carrying out clinical examination and other investigations. The serums PCT comes out to be a

better diagnostic test than serum CRP measurement as serum PCT was able to differentiate patients who came with abdominal pain but were having normal appendix from the patients who were actual cases of acute appendicitis. In patients with histologically confirmed acute appendicitis the level of PCT was above the normal value of 0.5 ng/ml in most cases. The analysis of procalcitonin in different groups of patients showed the serum procalcitonin test having high sensitivity of 95.65% and a specificity of about 100% on the basis of histopathological diagnosis of the removed appendix taking as the standard. The serum procalcitonin test when combined with reliable clinical signs and symptoms is an excellent diagnostic marker of the disease and should be done in the patients of pediatric appendicitis so that proper handling of the patient can be done and we can prevent unnecessary appendectomies.

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Introduction

Acute appendicitis is still the most frequent cause of right lower quadrant pain and the most common acute surgical condition of the abdomen. Acute appendicitis remains one of the most serious abdominal illnesses. It is a pathological condition that requires immediate surgical treatment. Its reported overall mortality rate is 0.3%, which rises considerably in cases of perforation (6.5%), older patients (5.5%) or neonates (80%) [1]. Numerous diseases mimic appendicitis, and it is often difficult to rule it out on the basis of clinical presentation. The frequency of appendicitis and the high rates of negative appendectomies, reported to be between 9 and 44%, have led to continuing efforts to

develop new diagnostic methods that have the highest possible sensitivity and specificity in order to reduce the rate of negative appendectomies [2]. Despite the multiple modern diagnostic tools that are available, diagnosis of acute appendicitis is still primarily done by history and physical examination. Additional diagnostic measures like temperature measurement, urinalysis and laboratory tests are routinely performed. Polymicrobial flora, predominantly, anaerobes, are usually found in an inflamed appendix. In recent studies, procalcitonin (PCT) has clearly been shown to be one of the most important biochemical indicators that closely correlate with the severity of the inflammatory host response to microbial infections [3]. Concentration of PCT selectively increases in case of bacterial infections, while in case of viral infections its concentration remains normal [4]. It has been seen that the level of procalcitonin correlates with severity of inflammation [5]. The diagnostic value of PCT as an investigation exceeds widely used parameters as fever, leukocyte count, erythrocytes sedimentation rate. It is a better marker than C-reactive protein [4, 6] which rise in inflammatory states. PCT can help in the diagnosis of pediatric appendicitis and can also prevent unnecessary appendectomies being performed in children in the age group of 0–15 years.

Material and Methods

The present study is a hospital based prospective observational study conducted in the “Post Graduate Department of Surgery, Government Medical College and Associated Hospital”. The study is approved by the Institutional Ethics Committee of Hospital and informed consent has been taken up when the case were seen. A total of forty patients were included in the study that were up to 15 years of age. Twenty eight patients were admitted with preliminary diagnosis of acute appendicitis. The control group involved around 12 healthy children within 3 to 15 years age group. The study was done for a period of one and a half years.

The patients whose appendix was normal after appendectomy were grouped into group I. Patients of acute uncomplicated appendicitis were grouped in group II and patients of acute complicated appendicitis (perforated appendix, gangrenous appendix, features of localized or generalized peritonitis) in group III. The control group (healthy children) was numbered as group IV.

All the patients were examined for signs and symptoms of disease on admission. A detailed history was taken and examination was done. Blood samples were collected. The investigations done on the patients were, body temperature, white blood cell (WBC) count, erythrocyte sedimentation rate (ESR), c reactive protein (CRP) levels, ultrasonography (USG) and computed tomography (CT) scan of abdomen/

pelvis (if required), depending solely on requirements. Emphasis was laid on WBC count, serum CRP levels and serum PCT levels in both, the diseased and control group. Serum PCT concentration was measured using the Immuno-luminometric Method (LUMI- Test PCT). The PCT level in healthy individuals was taken to be in the reference range of 0.1–0.5 ng/ml. Morphological investigation (histopathology) of appendix was also carried out. Statistical analysis was performed with the help of computer software SPSS 12.0.1 windows. The statistical significance was assessed by the use of one-way analysis of variance and by performing Fischer’s Protected “t” test. Diagnostic accuracy of PCT in classifying the subjects was assessed by the use of sensitivity, specificity and predictive values.

Observations

In this hospital based prospective observational study, forty patients upto 15 years of age were evaluated out of which 12 were controls; these controls were healthy children between 3–15 years of age group and were short-listed into Group I. The rest 28 patients who came to the emergency surgical ward with symptomology of acute abdominal pain were admitted and diagnosed clinically as acute appendicitis and thereafter operated for the disease. These patients were divided based on the morphological investigation of appendix, which was removed during the surgical intervention.

These patients were divided into three groups. The Group 1 involved 5 patients where after appendectomy the diagnoses of acute appendicitis was not conformed as the appendix removed was normal morphologically; the group II involved 12 Patients with morphologically confirmed diagnoses of acute uncomplicated appendicitis as the appendix was red, swollen and inflamed; the group III involved 11 patients with morphologically confirmed diagnoses of complicated form of acute appendicitis. The age sex distribution of patients is depicted in Table 1.

As tabulated in Table 2, the value of serum procalcitonin ranges between 0.09–0.18 ng/ml in control group. The same value in group I range between 0.14–0.21 ng/ml,

Table 1 Distribution of Patients according to age and sex (n=28)

Age	Boys		Girls	
	n	%	n	%
3–5 Yrs	2	4.14	0	0
5–10 Yrs	7	25	5	17.8
>10 Yrs	8	28.57	6	21.4
Total	17	60.71	11	39.29

Table 2 Serum PCT levels in (ng/ml) and CRP (mg/L) in different groups

	Group I (n=5) CRP ^a	Group I (n=5) PCT ^b	Group II (n=12) CRP ^a	Group II (n=12) PCT ^b	Group III (n=11) CRP ^a	Group III (n=11) PCT ^b	Group IV (n=12) CRP ^a	Group IV (n=12) PCT ^b
	10	0.21	18	0.59	35	0.82	2	0.08
	13	0.14	24	0.57	46	0.98	3	0.09
	8	0.18	20	0.65	40	0.95	2.5	0.12
	11	0.19	25	0.74	50	0.86	3	0.16
	12	0.14	12	0.67	49	0.90	1	0.18
			22	0.64	42	0.83	2	0.15
			17	0.63	48	0.96	5	0.10
			15	0.73	44	0.97	2	0.09
			26	0.48	41	0.90	1	0.12
			19	0.66	45	0.88	3	0.15
			28	0.63	44	0.85	3.5	0.09
			14	0.58			5	0.11
MEAN	10.8	0.17	20	0.63	44	0.90	2.75	0.12
SD ^c	1.92	0.0311	5.08	0.053	4.38	0.057	1.30	0.032

^a CRP Levels mg/L

^b PCT Levels ng/ml

^c SD - Standard deviation

while in group II the range is 0.48–0.74 ng/ml. In group III the range lies between 0.82–0.98 ng/ml. In this table, the value of serum CRP in control group ranges between 1–5 mg/L; in group I, it is between 8–13 mg/L. The value ranges between 14–28 mg/L and 35–50 mg/L in group II and III respectively. In patients with histologically confirmed acute appendicitis (in the group II and group III) the level of PCT was above the normal value of 0.5 ng/ml. In case of acute uncomplicated appendicitis (Group II) the mean level of PCT was (0.63 ng/ml). In patients with complicated appendicitis (group III), the mean level of PCT was 0.90 ng/ml.

Table 3 gives TLC counts in different groups as studied. Control group has range of 5000–13000/ml, group I has range of 7400–13800/ml, group II has range of 8600–15200/ml and group III has range between 5400–15400/ml. It has been revealed that in group I three patients (60%) had moderate leukocytosis and two (40%) had left shift. The CRP values in group I show an average of 10.8 mg/L (in the control group 2.75 mg/L). At the same time, level of procalcitonin in patients was not significantly different in these two groups compared to the normal value (0.17 ng/ml in group I and 0.12 ng/ml in control group). The moderate leukocytosis and left shift was detected in 6 patients (50%)

Table 3 TLC^a levels in different groups

	Group I (n=5)	Group II (n=12)	Group III (n=11)	Group IV (n=12)
	12500	11800	8400	5000
	8000	9900	11500	6500
	13800	13400	13900	10800
	7400	10900	6500	13000
	11600	13500	9800	7000
		14000	12400	5500
		9500	5400	8200
		11000	10700	9600
		15200	14200	9900
		8600	10100	11200
		12900	15400	8000
		14100		7500
Mean	10660	12066	10754.54	8516.66
SD	2820.99	2090.81	3166.50	2447.8

^a TLC/ml

in the group II and in 5 patients (45.5%) in group III. The average CRP values in-group II and III were around 20 mg/L and 44 mg/L respectively.

The Sensitivity, Specificity and Predictive values of serum PCT levels as the diagnostic test for the diagnosis of acute appendicitis:

Sensitivity = 95.65%

Specificity = 100%

Positive Predictive Value = 100%

Negative Predictive Value = 83.3%

Statistical inference of serum PCT levels as evaluated after Mean SD:

F value = 630.57

P value < 0.0000

As evaluated by Post test after one way ANOVA, the intergroup comparisons are tabulated after evaluation through statistical analysis in Table 4. Only the comparison between Group II and Group I is statistically insignificant ($p=0.04$). In the comparison between the rest of the groups like group IV and III, group III and II etc; all the intergroup comparisons show statistical significance ($p<0.00001$).

Discussion

PCT is a calcitonin precursor and has emerged as one of the most important early laboratory signs for systemic bacterial and fungal infection. The group of calcitonin precursors, including PCT, are encoded by the calcitonin I gene (CALC-I) on chromosome 11 [7]. In the absence of inflammatory stimuli, the transcription of the CALC-I gene is suppressed, except in neuroendocrine cells that are mainly found in the thyroid or lung. In healthy individuals, the normal plasma PCT levels are <0.5 ng/ml. In cases of microbial infection, severe systemic inflammation or sepsis, an increase in CALC-I gene expression occurs, with a concomitant rise in PCT levels in all tissues and cell types of the human body [8]. In many cases, the acute

appendicitis has atypical course that makes difficult to diagnose timely, which delays surgical intervention. Nowadays we have no laboratory parameters that could indicate or reliably point on presence of acute appendicitis. One possible diagnostic parameter of this surgical pathology is the plasma protein – PCT. PCT is considered as the marker of bacterial infections [4, 6].

From a pathophysiological point of view, fecaliths, which naturally carry a high load of gram-negative bacteria, are thought to be the predominant factor for the obstruction of the appendiceal lumen in acute appendicitis. Over the course of the inflammatory process, mucosal secretion increases and the resident bacteria of the appendix start to multiply rapidly. The integrity of the appendix is compromised through an impaired blood supply, and the bacterial invasion begins to proceed with the release of bacterial endotoxins [9]. At the same time, it has been demonstrated that the injection of bacterial endotoxin into healthy subjects causes an increase in PCT by approximately 0.5 ng/ml per hour after a latency of about 2–3 hours and reaching a plateau after 6–12 hours [10]. Bacterial lipopolysaccharides are unique surface glycolipids and prototypical examples of bacterial endotoxins, which are found in the outer membrane of gram-negative bacteria. Bacterial lipopolysaccharides and the proinflammatory cytokines are the most potent inducers of PCT release. During a viral infection, the serum PCT concentrations were slightly elevated to 1.5 ng/ml, whereas the levels during a bacterial infection reached up to 1,000 ng/ml [11]. Therefore, an increase in PCT in patients with acute appendicitis can be expected.

A useful biomarker for sepsis must be Specific (as well as Sensitive), Measurable with a high degree of precision, readily Available (and Affordable), Responsive and Reproducible, with results available in a Timely fashion to guide therapy (SMART). PCT fulfills most of the criteria for a SMART biomarker. The specificity and negative predictive value of PCT have been substantially enhanced in newer assays by the improved functional assay sensitivity of an amplified cryptate emission technique [12, 13]. The

Table 4 Intergroup comparisons as evaluated by post test after one way ANOVA

Group IV Vs Group III	T=13.48 P<0.00001	Statistically significant
Group IV Vs Group II	T=28.4998 P<0.00001	Statistically significant
Group IV Vs Group I	T=39.45 P<0.00001	Statistically significant
Group III Vs Group II	T=18.3002 P<0.00001	Statistically significant
Group III Vs Group I	T=26.55 P<0.00001	Statistically significant
Group II Vs Group I	T=2.06 P=0.04	Statistically insignificant

biostability of PCT makes it measurable within a realistic clinical window with an assay that is available and relatively affordable. PCT is responsive and reflects the severity of the disease process and effectiveness of therapeutic interventions [14]. With a half-life of about 24 hours, it allows for timely repeated measurements that may reflect changes of the underlying clinical condition due to therapy. The cost of procalcitonin measurement is US\$10 to \$30 per sample [15] and the potential savings in the consumption of other health-care resources by preventing unnecessary appendectomies is evident.

The serum PCT comes out to be a better diagnostic test than serum CRP measurement [4, 6, 16] as serum PCT was able to differentiate between group I patients (who came with abdominal pain but were having normal appendix) from group II and group III patients who were actual patients of acute appendicitis. The mean CRP level in group I (10.8 mg/L) was slightly higher than the normal CRP ranges (0–10 mg/L) and hence could not differentiate group I from group II and III while the mean serum PCT level in group I was 0.17 ng./ml which was lower than the normal serum PCT level (<0.5 ng./ml).

Importance of PCT as nonspecific marker of inflammation has been stated in case of various bacterial infections, sepsis shock, and acute pancreatitis [4, 6, 16–18]. As seen by the observations, analysis of PCT in different groups of patients as shown the serum PCT test has a high sensitivity of 95.65% and a specificity of about 100% on the basis of morphological diagnosis (histopathology) of the appendix taking as the standard. The positive predictive value was around 100% and negative predictive value about 83.3%. Only one false negative case was reported. The statistical inference of serum PCT levels also shows ‘f’ value of 630.57 and ‘p’ valued of <0.00001 (statistically significant). The intergroup comparison also proves the statistical significance of this test. The available data evaluating the diagnostic value of PCT for patients with acute appendicitis are sparse and predominantly related to children. Kafetzis et al. [19] showed that PCT value of >0.5 ng/ml was found to be indicative of perforation or gangrene of appendicitis with 73.4% sensitivity and 94.6% specificity.

Thus, analysis of PCT in different groups of patients has shown that increase in PCT level correlate with disease severity [5, 16] statistically and significantly differs between the groups and maximally increases in case of acute complicated appendicitis.

Conclusions

The serum PCT test was found to be a better indicator for diagnosing the condition than the serum CRP and other tests. On comparing the results, the serum PCT is a more

sensitive indicator than serum CRP and other tests not only to diagnose the disease but can also predict its severity.

The serum PCT test when combined with reliable clinical signs and symptoms is an excellent diagnostic marker of the disease and should be done in the patients of pediatric appendicitis so that proper handling of the patient can be done and we can prevent unnecessary appendectomies. Also, surgical explorations and prediction of complications can be done depending on its quantities value i.e. the more higher the value, the severity of disease is more and henceforth complications can be expected.

Not only this, as shown by researches going on from the last decade, PCT is now emerging as a good biomarker for other inflammatory conditions too and some clinical trials have shown it to differentiate between infections of bacterial and non bacterial origin too. We can conclude that PCT can be used as a very good diagnostic marker for acute appendicitis – this marker in case of acute appendicitis would help us to carry out timely and indispensable surgical interventions and predict disease complications.

A child who comes to emergency surgical ward with acute lower abdominal pain can undergo serum PCT measurement in addition to clinical symptoms and signs before being operated upon for acute appendicitis directly. If serum PCT level comes out to be normal we can exclude the possibility of acute appendicitis while if it comes out to be raised then we can give a possible diagnosis of acute appendicitis keeping in view the clinical signs and symptoms of the disease whilst the other inflammatory conditions being ruled out.

Conflicting Interest Nil

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