The Effect of Time Interval from Index Trauma on Results of Diagnostic Peritoneal Lavage in Animal Samples

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Abstract: Diagnostic peritoneal lavage (DPL) is an invasive and highly sensitive diagnostic tool to detect abdominal trauma. Despite recent trend to use non-invasive tools such as FAST and CT scan to detect these injuries, DPL has its place in trauma management particularly for hollow visceral injury. Using RBC count to detect intestinal injuries has had low accuracy rate. WBC count, amylase, and alkaline phophatase (ALP) levels have been useful in this regard but with some controversies. The study was carried out on 90 rats, divided to 3 groups (30 rats each), and every group was subdivided randomly to 15 case and 15 control rats. For case rats, perforations were done in the small intestine using a needle. In all rats a catheter was placed in the peritoneum. Lavage of the peritoneum was done through the catheter 1, 6, and 12 hours after the trauma for first, second, and third group, respectively. WBC count was not different between case and control rats 1 hour post-injury, but was increased 6 and 12 hours after perforation in case rats. Amylase and ALP levels were higher in case rats in all of three groups compared to control rats. Further human studies must be done to clarify the normal and abnormal values of WBC count, amylase, and ALP levels of DPL analysis. Furthermore, the effect of time interval on these parameters must be appreciated and taken into account in interpreting the results of DPL to detect abdominal injuries as soon as possible.
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1. Introduction

Diagnostic peritoneal lavage (DPL) is an invasive procedure to detect intra-abdominal injuries (Nagy et al., 2000). It is highly sensitive in detecting intra-peritoneal bleeding, and has been used in blunt and penetrating anterior abdominal trauma in the past 3 to 4 decades (Root et al., 1965; Fischer et al., 1978). It is also can be used to detect diaphragmatic injury in thoraco-abdominal penetrating trauma. However in recent years FAST ultrasound and abdominal CT scan using multidetector devices are replacing DPL to detect intra-abdominal injuries (Ozturk et al., 2004; Myers et al., 2000; Malhotra et al., 2000). FAST is a non-invasive procedure that can be done on the bedside and with minimal training can be as sensitive as DPL. CT scan is sensitive and specific in detecting intra-abdominal injuries, and can detect retroperitoneal injuries that DPL will miss. Despite these changes (Bhan et al., 2007), DPL has its place in trauma management and is recommended in the last edition of ATLS (ACS Committee on Trauma, 2012).

Analyzing DPL results has been controversial to some degree (Sato et al., 2005). Factor such as RBC count or the presence of intestinal contents are the most agreed on (Whitehouse and Weigelt, 2009). WBC count, amylase, and alkaline phophatase enzyme levels are more controversial and some authors questioned their usefulness (Jaffin et al., 1993; McAnena et al., 1991; Megison and Weigelt, 1990; McAnena et al., 1991). Concerning that most of current indications for DPL involve detecting hollow visceral injury which cannot be detected reliably by RBC count, it seems necessary to further clarify the characteristics of other parameters in DPL results. This experiment is to study the effect of time interval from a hollow visceral injury on WBC count, amylase level, and ALP level of DPL analysis results in an animal model.

2. Material and Methods

The study was carried out on 90 male rats (Albino Wistar type) without any history of trauma, ranging in weight from 190 to 210 g, which were obtained from Pasteur Institute of Iran. They were randomly designated to 3 groups (30 rats in each group), and every group was subdivided randomly to 15 case and 15 control rats.

All rats were sedated with ketamine and peritoneum was opened using a 1 cm incision. In every group, for case rats, 3 through and through perforations were done in the small intestine using 21 gauge needle. For control rats no further trauma was done. In all rats a catheter was placed in the

peritoneum to do the lavage later, and the abdominal incision was repaired.

In the first group lavage of the peritoneum was done through the previously placed catheter 1 hour after the trauma. In the second group this was done 6 hours after the trauma, and in the third group the time interval was 12 hours. Peritoneal lavage was done using 20 ml sterile saline, and the effluent was analyzed for white blood cell (WBC) count, amylase, and alkaline phosphatase (ALP) levels. Measurements were done blindly in the laboratory.

All these rats were housed in an air-conditioned animal room at an ambient temperature of 23°C, relative humidity ranging from 55% to 60%, and a 12-h (on/off light) cycle. All animal experimentation protocols were approved by the Ethical Committee of Tabriz University of Medical Sciences and conformed to the principles outlined in the Declaration of Helsinki.

Data analysis was done by the SPSS version 16 software. Mean and standard deviation (SD) were calculated for each group and subgroup, and student's t-test was used for statistical analysis. P-value of less than 0.05 was considered to be significant.

3. Results

Mean WBC count of DPL effluent in control rats was 5.93, 6.93, and 7.33 per cm3 in the first, second, and third groups respectively. Mean amylase level for control rats was 1.14, 1.04, and 1.14 IU/L for 1, 6, and 12 hours time intervals respectively. Mean ALP level for control rats was 1.97, 1.83, and 1.66 IU/L in the first, second, and third groups respectively.

Mean WBC count of DPL in rats with intestinal perforations was 6.20, 77.26, and 114.47 per cm3 for 1, 6, and 12 hours time intervals respectively. Mean amylase level for case rats was 2.14, 6.99, and 18.33 IU/L in the first, second, and third groups respectively. Mean ALP level for injured rats was 3.46, 14.03, and 29.46 IU/L for 1, 6, and 12 hours time intervals respectively.

WBC count means of DPL were not different for case and control rats in the first group, with 1 hour time interval (P=0.81), but there was statistically significant rise of WBC count for the intestinal perforation cases compared to control rats in second and third groups, with time interval of 6 and 12 hours, respectively (P<0.001). For the amylase levels, there was a statistically significant rise in case rats compared to control rats in all groups. Also for the ALP levels there was a statistically significant rise from the first hour following the intestinal perforation. These data are summarized in Table 1.

Table 1. Results of laboratory analysis of DPL in case and control rats 1, 6, and 12 hours after intestinal perforation (WBC, white blood cell; ALP, alkaline phosphatase; Amylase and ALP in (IU/L))

Group	Variable	Case	Control	P value
First group	WBC/cm^3	6.2±2.27	5.93±3.59	0.81
	Amylase	2.14±0.99	1.14±0.54	0.002
	ALP	3.46±1.59	1.97±0.36	0.001
Second group	WBC/cm^3	77.26±20.32	6.93±3.21	<0.001
	Amylase	6.99±2.27	1.04±0.58	<0.001
	ALP	14.03±4.62	1.83±0.63	<0.001
Third group	WBC/cm^3	114.47±31.5	7.33±3.90	<0.001
	Amylase	18.33±7.04	1.14±0.44	<0.001
	ALP	29.46±6.01	1.66±0.72	<0.001

4. Discussion

Despite current trend to use FAST and CT scan to diagnose intra-abdominal injuries in most trauma patients, DPL seems to be an undeniable diagnostic tool in these patients (Cha et al., 2009). Stable blunt trauma patient with intra-peritoneal fluid whom cannot be examined serially (due to decreased level of consciousness or being operated for other injuries) must undergo DPL. In anterior thoraco-abdominal stab wound patients without clear indication for laparotomy, DPL is a reliable diagnostic procedure to decrease rate of negative laparotomies (Hashemzadeh et al., 2012; Gonzalez et al., 2001; Sriussadaporn et al., 2002). And in places without ultrasound or CT scan capabilities, DPL can be done with minimal requirements.

Detecting hollow visceral injury by DPL can be done by finding intestinal content in the DPL effluent. Although this seems obvious, it cannot be found in all intestinal injuries and other parameters have been included in the analysis of DPL to raise the accuracy of the test. WBC count, amylase, and ALP levels have been used for this purpose. WBC count is more accepted than enzyme levels, and has been mentioned in the last edition of ATLS (ACS Committee on Trauma, 2012). Although even for the WBC count there has been some controversies, and DPL cell count ratio has been introduced by Fang et al (1998) to address some of these problems (Wang et al., 2012). Jaffin et al (1993) recommended using ALP levels only in the management of patients with equivocal findings on DPL who would otherwise not undergo laparotomy. Mc Anena et al (1991) found elevated DPL amylase and ALP levels correlated with the presence of intestinal perforations. However, Megison et al (1990) questioned usefulness of determining ALP levels in DPL effluent.

In this study we developed an animal model to assess the effect of time interval from initial intestinal injury to the DPL procedure. WBC counts

were not elevated in the first hour post injury, but they were elevated in 6th and 12th hours. This can be explained by considering the fact that the migration of WBCs to the peritoneum takes time. Amylase and ALP levels in DPL effluent were elevated in 1st, 6th, and 12th hours, and showed an increasing trend for both enzymes in passing hours. These findings probably can be explained by considering that amylase originate from intraluminal intestinal fluid and is present just after the injury. Likewise, ALP has been assumed to originate from intestine and is present from the beginning.

There are some inherent limitations in applying these finding to human subjects, but our findings warrant further human studies to clarify the normal and abnormal values of WBC count, amylase, and ALP levels of DPL analysis. Furthermore, the effect of time interval on these parameters must be appreciated and taken into account in interpreting the results to detect abdominal injuries as soon as possible.

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