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APPRAISAL OF OXIDATIVE STRESS IN OPEN VERSUS LAPARASCOPIC CHOLECYSTECTOMY

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ABSTRACT

Cholecystectomy is one of the most common surgeries in the operative world. The choice between open and laparoscopic procedures is a major point of decision taking among surgeons. This study aims to evaluate the effect of oxidative stress on these procedures and how it influences the course of these procedures. Out of 50 study subjects 25 were operated on by open and 25 by laparoscopic procedures and the total antioxidant (TAS) and thiobarbituric acid reactive substances(TBARS) were assayed preoperatively, 90 mins and 12 hours postoperatively. It was found that the level of TBARS was significantly higher in open cholecystectomy in all three temporal profile and TAS was lower in open cases.

Keywords: Oxidative Stress, Total Antioxidant Status, Thiobarbituric Acid Reacting Substances, Cholecystectomy, Tbars

Introduction:

Cells under aerobic condition are always threatened with the insult of ROS which however efficiently taken care by antioxidant systems of our body as 5% or more of the inhaled O2 is converted to reactive oxygen species (ROS), such as O.2, H2O2, OH. by univalent reduction of oxygen. If the generation of ROS exceeds the capacity of the antioxidant defenses the oxidative stress results. The ultimate effect of oxidative stress is the damage to all types of biomolecules including DNA, proteins and lipids. The primary target depends upon type of cells, type of stress imposed and severity of stress. In acute cholecystitis elective laparoscopic cholecystectomy is now established as the treatment of choice for symptomatic subjects (1). The oxidative stress markers are elevated in surgical procedures due to ischemia/reperfusion injury due to variation of intra-abdominal pressure on account of generation of pnemoperitoneum (2). Immediately after cholecystectomy they will rise to a certain extent and hours after post operative period a further rise can be noticed. But an increased rate of rise of such markers can be early predictors of complications where inflammatory tissue injury remains in action. However in post operative period of open cholecystectomy these indicators are more elevated than laparoscopic cholecystectomy indicating the choice of surgery also.

Materials and methods:

The objectives of the study are to assess the variation of values of the indicator after cholecystectomy, to assess the role of these parameters as early predictor of post operative complications, to prevent the possibilities of oxidative tissue damage in the process of operative management of cholecystitis in future. Patients were selected from Surgical Out Patient Department (OPD) of N.R.S. Medical College and Hospital. A comparative study was undertaken in the department of Surgery in collaboration with Department of

Biochemistry, N. R. S. Medical College & Hospital, Kolkata, West Bengal. The study was conducted in patients of chronic calculous cholecystitis. Patients selected for the study were those who were symptomatic for last 6 months, with no history of jaundice, USG showed GB stone with normal CBD diameter and absence of calculi in CBD, LFT reports were within normal limits. Patients excluded from the study were those having stone in biliary tree other than gall bladder, having acute inflammatory symptoms, having empyema, mucocele or carcinoma of gall bladder. 50 subjects of chronic calculus cholecystitis were selected after a through screening by maintaining our inclusion criteria. These subjects were than randomly planned for open and laparoscopic cholecystectomy having 25 subjects in each group. Outcome of the study was compared between the two groups.

Parameters studied:

1) Outcomes of the two different modes of surgical procedures will be studied biochemically after 90 minutes and 12 hours in comparison to preoperative values.
2) Sonological and biochemical parameters to include and exclude patients were performed as discussed above.

Study tools:

1) Thiobarbituric Acid Reactive Substances (TBARS) estimations.
2) Total Antioxidant Status ((TAS) estimation.

Estimation of total antioxidant status:

Methods that have developed for the measurement of the total antioxidant activity of fluid are all essentially inhibition methods. A free radical species is generated, there is an end point by which the presence of radical is detected and the antioxidant activity of the added sample inhibits the end point by scavenging the free radical. This study used the method proposed by Rice Evans and N. J. Miller (methods in ENZYMOLOGY, 1994, vol. 234, 279-293) used (3). Method of measuring total antioxidant activity is based on the inhibition of absorbance of the radical cation of 2,2/ – azinobis, 3–ethylbenzothiazoline 6 sulfonate ($ABTS^{\bullet+}$) which has a characteristic long-wavelength absorption sprectum at 734 nm by antioxidants present in serum against a known standard antioxidant Trolox (6 – hydroxyl- 2, 5, 7, 8 – tetramethylchroman –2 – carboxylic acid). $ABTS^{\bullet+}$ radical
cation is formed by interaction of ABTS with ferryl myoglobin radical species generated by activation of metmyoglobin with H_2O_2. Antioxidants present in serum suppress the absorbance of the $ABTS^{\bullet+}$ radical cation.

Reagents used:

TROLOX (2.5 mM), 5mM PBS (pH 7.4), ABTS , H_2O_2 (450 μM), Metmyoglobin.

Estimation of serum thiobarbituric acid reactive substance (4)**:**

Thiobarbituric acid (TBA) when allowed to react with MDA (malondialdehyde) aerobicaly form coloured complex (pink colour) which is measured calorimetrically at 532 nm. MDA is extracted into TCA (tricarboxylic acid) & coupled with TBA, MDA produces pink colour when couple to TBA. This colour is extracted into n- butanol & measured. Reagents used were 20% TCA, 0.05 (M) H_2SO_4, 2 M Sodium sulphate, % Thiobarbituric acid, n Butyl Alcohol and standard solution of MDA.

Result and Analysis:

In this study 50 cases of chronic calculous cholecystitis were taken as study group. They were randomly divided into 25 open cholecystectomy (OC) and 25 laparoscopic cholecystectomy (LC) groups.

Thiobarbituric Acid Reactive substances (TBARS) preoperatively:

	GROUP	N	Mean	Std. Deviation	Std. Error Mean	't' value	Sig. (2-tailed)
TBARS	OC	25	5.4080	0.09967	0.01993		
	LC	25	5.3560	0.13565	0.02713	1.545	0.130

Above statistical data shows that there is no statistical significant difference between preoperative values of TBARS in both groups (p=0.130 i.e. p>0.05).

TBARS after 90min and 12 hr postoperatively

	GROUP	N	Mean	Std. Deviation	Std. Error Mean	't' value	Sig. (2-tailed)
TBARS_90	OC	25	7.3480	.22383	.04477	17.512	0.000
	LC	25	6.1120	.27282	.05456	14.049	0.000
TBARS_12	OC	25	8.5720	.34943	.06989	14.049	0.000
	LC	25	6.8560	.50090	.10018		

Statistical analysis shows differences TBARS values at two different post operative period among the OC and LC patients are statistically significant (both $p<0.05$) and mean values of TBARS in OC patients are greater than that of LC patients.

Total Antioxidant status (TAS) preoperative, early (90mnts.) and late (12hrs) post operative.

	GROUP	N	Mean	Std. Deviation	Std. Error Mean	't' value	Sig. (2-tailed)
TAS_pre	OC	25	1.3480	.18285	.03657		
	LC	25	1.4280	.14000	.02800	-1.737	.089
TAS_90	OC	25	1.0560	.16852	.03370	-7.150	.000
	LC	25	1.3720	.14295	.02859		
TAS_12	OC	25	.6640	.10755	.02151	-11.214	.000
	LC	25	1.1680	.19732	.03946		

Above statistical data shows that there is no significant difference between pre operative values of TAS in both groups ($p=0.089$ i.e. $p>0.05$). Statistical analysis shows differences TAS values at two different post operative period among the OC and LC patients are statistically significant (both $p<0.05$) and mean values of TAS in OC patients are less that of LC patients.

Discussion:

In our study though patients of chronic cholecystitis were participated but there was no scope of comparing oxidative stress markers to healthy volunteers. Preoperative values of both the markers in two groups have been compared to avoid any bias.

Both the result of comparative analysis of preoperative values of Thiobarbituric Acid Reactive substances (TBARS) and Total Antioxidant Status (TAS) between open and laparoscopic group are insignificant (TBARS: $p=0.130$, TAS: $p=0.089$ in both cases

p>0.05).

It has been reported that injuries and operations can induce oxidative stress. Both traditional open cholecystectomy and laparoscopic cholecystectomy cause oxidative stress and lead to an antioxidant response with the release of various inflammatory mediators including cytokines, arachidonic acid metabolites, complement products, lysozomal enzymes, and oxygen free radicals. Recent studies have suggested interleukin-6 (IL-6) as a marker of severity of injury and as a predictor of major complications. Cruickshank at al. confirmed the association between the degree of surgical injury and the magnitude of the IL-6 response (5). Reilly PM, Bulkley GB (1990) commented that the increase in lipid peroxidation is inevitable and so the risk of cellular damage is high in surgical patients (6). In the present study evaluation and comparison of values of Thiobarbituric Acid Reactive substances (TBARS) and Total Antioxidant Status (TAS) preoperatively, 90min after incision and 12hr after incision separately in OC and LC group (paired in-group data study) give significant result in both the groups.

In case of OC, mean value TBARS preoperatively (5.408) < mean value TBARS after 90 min (7.348) < Mean value TBAR after 12hr (8.572) (in all case p<0.05).

In case of LC, mean value TBARS preoperatively (5.356) < mean value TBARS after 90 min (6.112) < mean value TBARS after 12hr (6.856) (in all case p<0.05).

In case of OC, mean value TAS preoperatively (1.348) > mean value TAS after 90 min (1.056) > mean value TAS after 12hr (0.664) (in all case p<0.05).

In case of LC, mean value TAS preoperatively (1.428) > mean value TAS after 90 min (1.372) > mean value TAS after 12hr (1.168) (in all case p<0.05).

These values being statistically significant, definitely agree with the fact that whether by open or laparoscopic approach, the definitive surgery induces oxidative stress response resulting rise of oxidative stress factors and simultaneous stimulation of anti-oxidative response. It is clearly evident that tissue injury during open approach causes oxidative stress. Gal et al 1998 investigated surgical trauma induced by laparoscopic cholecystectomy and showed both open and laparoscopic procedures induced changes in acute phase response, free radical mediated reactions and neutrophil function (7). Our result is also consistent with the result of Glantzounis et al 2001 who concluded that free radical induced lipid peroxidation resulted in decreased TAS and uric acid level after deflation of pneumoperitonium (8).

Several studies have been conducted reflecting the comparison of oxidative stress evolved as result of open (OC) and laparoscopic cholecystectomy (LC).

Schietroma et al 2001 evaluated that LC has less effect on oxidative stress than OC during surgery (9). Zulfikaroglu et al 2002 have shown that antioxidant defense system is stimulated less in LC than OC (TAS was one of the important marker in their study (10). TAS p<0.01) reflecting LC as more safe technique. On the other hand, Seven et al 1999 examined lipid peroxidation and antioxidant state after open and laparoscopic cholecystectomy. They concluded that LC cause significant less oxidative stress than OC (11). Gal et al 1998 also showed same result in favour of LC (7). In a study conducted by Bukan et al in Ankara, Turkey 2004, they have shown significant difference (p<0.05) of post operative values of oxidative stress markers (nitrite + nitrate and MDA) between LC and OC resulting a less post operative oxidative stress in LC (1).

In our study the comparative analysis of post operative values of TBARS and TAS at 90 min and 12 hrs after incision yield a significant change in both the values (all cases p<0.001). In case of TBARS, mean value after 90min in OC (7.348) > mean value after 90min in LC (6.112) and mean value after 12 hr in OC (8.572) > mean value after 12 hr in LC (6.586). Also in case of TAS mean value after 90min in OC (1.056) < mean value after 90min in LC (1.372) and mean value after 12 hr in OC (0.664) < mean value after 12 hr in LC (1.168).

These values effectively say that there is increased oxidative stress in OC than in LC as pro-oxidative stress marker TBAR increased more in OC and counteracting anti-oxidative stress marker TAS decreased more in OC than LC. The changes are statistically significant revealing laparoscopic approach (LC) to be a safer one.

Although Zulfikaroglu et al took operating time as single independent parameter and compare it in both OC and LC groups but they have not shown any analysis regarding the relationship between operating time and oxidative stress markers.

In our study there is a positive correlation between TBARS after 90min and operating time and negative correlation between TAS and operating time as evident from Pearson Correlation test. So it can be hypothesized that increasing operating time also can contribute to increased oxidative stress though further study including large samples is needed.

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