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Alteration of Gene Expression Due to Arsenic Induced Oxidative Stress Leading to Ovarian Cancer

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Abstract
Epidemiological studies and preventive measures for cancer have engaged most of the scientists and doctors for several decades and the research has come forth a long way now. Several heavy metals like arsenic have been linked with different types of cancer, one of which is ovarian cancer (Oca). Higher mortality rate of Oca signifies the gravity of the case. Moreover, arsenic is also believed to invoke generation of free radical and the consequences are even exacerbated. Early diagnosis of Oca is presently quite feeble and CA-125 alone seems to be insufficient and capricious. However, CA-125 along with other diagnostic parameter satisfies most of the pathologists and clinicians. For determination of oxidative stress, Malondialdehyde (MDA) was chosen as the marker and arsenic estimation was performed with the help of Atomic Absorption Spectrometer (AAS). RBC count, Heamoglobin, SGPT, SGOT and Alkaline phosphatase levels were performed according to standard protocol. Higher level of arsenic increases MDA level, decreases CA-125 level, decreases RBC and Haemoglobin level and increases SGPT, SGOT, ALP levels and vice versa for no arsenic in the Oca patients with significance <0.0001, <0.001, <0.005, <0.020, <0.001, >0.05, and <0.002 respectively. Concludingly, arsenic is one of the major causative agents for oxidative stress and hence leading to cancer including Oca. CA-125 was estimated to be very higher in Oca patients than normal ones and RBC count, Heamoglobin, SGPT, SGOT, ALP levels also seemed to be varied from normal.
Keywords- *Arsenic, MDA, CA-125, RBC, WBC.*

1. INTRODUCTION

Deadliness of ovarian cancer is now evident as it ranks fifth in cancer deaths among women. It has been estimated that risk of conceiving ovarian cancer during a woman's life is about 1 in 73 and her chance of dying of ovarian cancer is about 1 in 100. Ovarian cancer has been classified on the basis of its origin: (a) surface epithelium derived from either the coelomic epithelium or ectopic endometrial epithelium, (b) germ cells, which migrate unto the ovary from yolk sac and are totipotential, and (c) the stroma of the ovary, which include sex chords. Most primary types of neoplasm in the ovary are categorized under the tumour of mŭllerian epithelium. The three major types of tumours of epithelial origin are serous, mucinous, and endometroid.

CA-125 is a transmembrane glycoprotein embedded on plasma membrane of cells that are transformed to metaplastic to give rise to mŭllerian epithelium [1]. CA-125 is still the most extensive studied biomarker and adopted worldwide for early detection of Oca [2]. But on the other hand, elevated level of soluble CA-125 has been detected in other malignant cancers such as breast cancer [3], mesothelioma, non-hodgkin lymphoma, gastric cancer, and in other benign conditions too, such as endometriosis [4], pregnancy, ovulatory cycle, liver diseases and congenital heart failure as well as infectious diseases like tuberculosis.

Lipid peroxidation is perpetually occurring in cells as normal cellular metabolism. LPO is one of the most extensively studied consequences of Reactive Oxygen Species (ROS), perturbing the integrity of the plasma membrane and the function of the cell. Polyunsaturated Fatty Acids (PUFAs) are extremely susceptible to peroxidation. The inkling that lipid peroxides and ROS participate in inextricable signal transduction cascade responsible for the control of the cell proliferation, and the induction of differentiation, maturation and apoptosis has been corroborated by several literature [5]. LPO mediated oxidative stress can sabotage major cellular components and function like DNA strand breakage, rises in intracellular free ca2+ , damage to membrane ion transporters and/or specific proteins [6]. MDA, an aldehyde product of LPO has been established to possess mutagenic and carcinogenic effect. It appears to bind with bases dG, dC and dA to build m1G, m1C and m1A respectively [7].

Arsenic sits at 33rd position on the periodic table of chemical elements and it is categorized as a metalloid, however it is most frequently referred as heavy metal as in context of toxicology [8]. A number of results provide evidences that toxic and carcinogenic metals like arsenic has capabilities to bind with different nuclear proteins and DNA causing oxidative deterioration of biological macromolecules. Arsenic exposure has been established to be one of the factors for generation of ROS [9]. Accumulation of arsenic in greater quantity in the body has debilitating impact on overall human health and has also been implicated in cancer.

In our current investigation, correlation between MDA, CA-125, Haemoglobin levels and RBC count have been attempted to establish and

besides that, alkaline phosphate, SGOT, and SGPT levels provides panoptic view to this study.

2. MATERIALS and METHODS

The blood samples were collected intravenously from the 110 ovarian cancer patients who came for the treatment in Mahavir Cancer Institute and Research Center, patna and 48 healthy women of comparable age group, with their consent. Part of blood was used for RBC count, estimation of Haemoglobin level and arsenic concentration and serum was prepared from rest of blood for the analyses of SGPT, SGOT, Alkaline Phosphotase, and MDA levels.

2.1 Haematological study

RBC count and Haemoglobin level were performed by standard protocol using Cell Counter (Medonic M- Series) model no- 18253.

2.2 MDA Assessment

Serum of OCa patients was prepared by centrifuging blood sample at 3000g for 10 minutes. LPO assay was performed by a standard protocol with slight modification [10].

2.3 ALP, SGPT and SGOT

Serum was used for liver function test which included ALP, SGOT, and SGPT. Liver function test was analysed by auto-analyzer (Vitalab-selectra pro XL) model no- 8-7136.

2.4 CA-125 assay

CA-125 was estimated from the serum with the help of ELISA kit method.

2.5 Estimation of Arsenic

1ml of blood was transferred to EDTA vials of randomly selected 24 out of 110 ovarian cancer patients for estimation of arsenic concentration. Further, sample preparation and estimation of arsenic was done by standard protocol of Atomic Absorption Spectrometer graphite flame (Perkin Elmer) model number 900T.

2.6 Statistical analysis

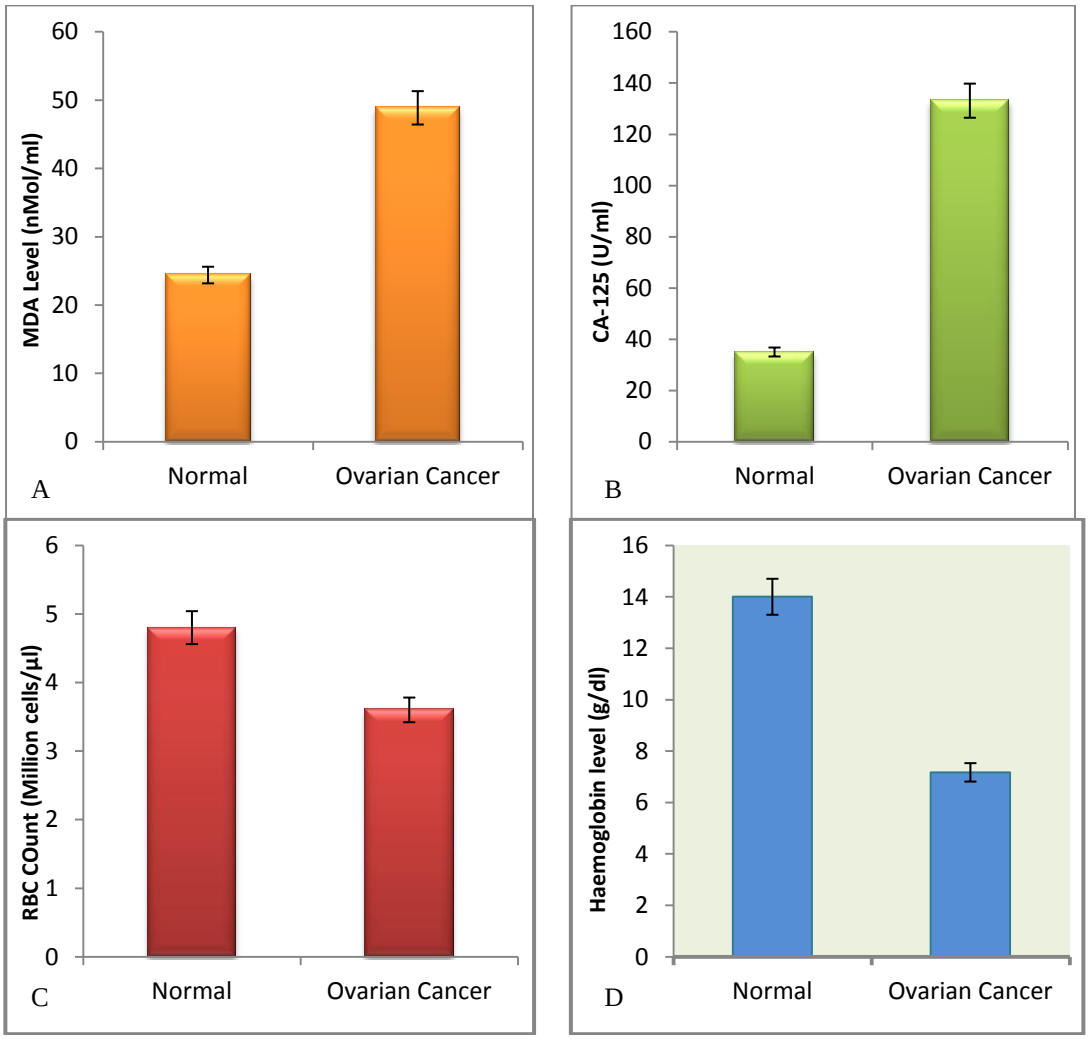
Mean±SD and p-value were obtained using SPSS software (statistical package for social sciences, version 16.0). A p-value less than 0.05 were considered significant.

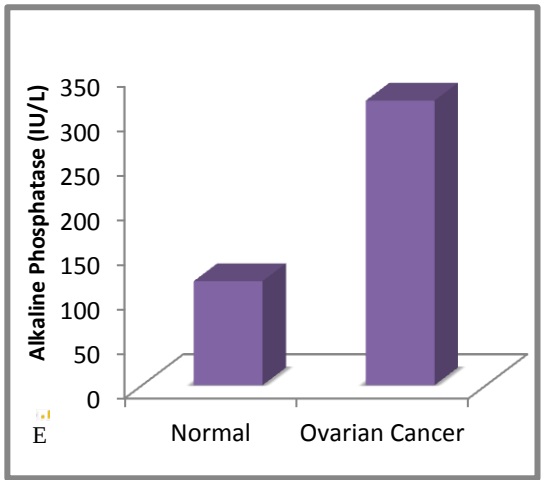
3. RESULTS

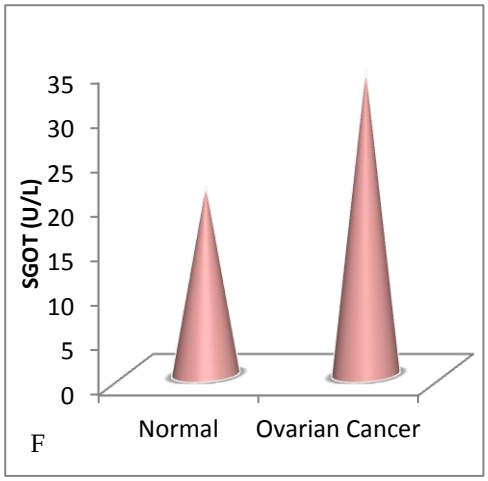
As per calculations, Mean±S.D of MDA level (nMol/ml) in ovarian cancer patients and normal persons was found to be 48.87±11.97 and 24.39±13.39, respectively with p-value <0.0001. Text fig 1(A) demonstrates the comparative hike in MDA level in ovarian cancer patients than normal persons. Conventionally, CA-125 level in ovarian cancer patients is higher than normal persons, espousing quite an orthodox pattern. Mean±S.D of CA-125 (U/ml) in ovarian cancer patients was 133.11±293 and that of normal persons was 31.81±9.56 with significance level <0.0024, as depicted in text fig 1(B).

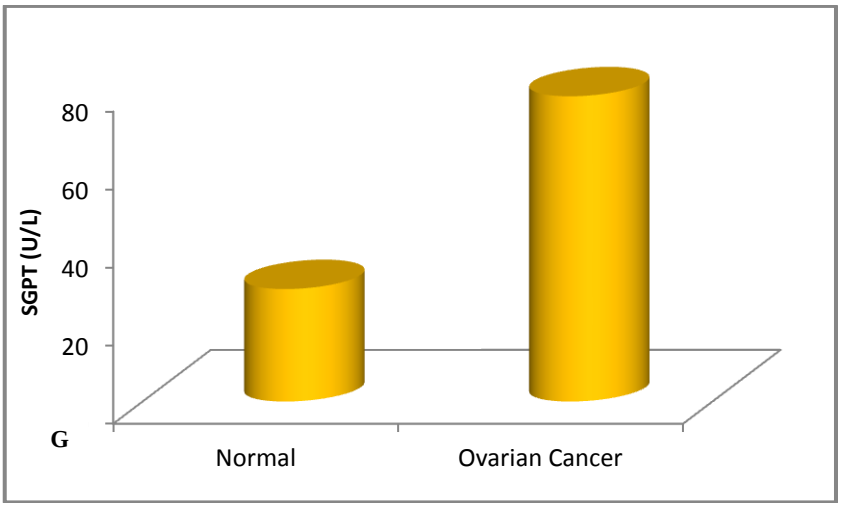
On the other hand, mean±S.D of haemoglobin level (g/dl) in ovarian cancer patients (7.17±3.75) was obtrusively lower than healthy women (13.106±1.98) with p-value <0.001 as shown in text fig 1(C). Whereas red blood cells count in ovarian cancer patients and healthy women yielded noticeable difference as seen in text fig 1(D). Mean±S.D of RBC count in ovarian cancer and normal women was calculated to be 3.6±0.82 and 6.1±1.7, respectively which has significance value <0.001.

There were tremendous variations in several enzymes levels like alkaline phosphatase (ALP), serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT), in ovarian cancer patients and healthy persons. Mean±S.D of ALP level (U/L) in ovarian cancer patients was higher (319.96±100.07) than that of healthy ones (117.45±39.67) with significance <0.005 as plotted in text fig 1(E). Text fig. 1(F) showss that Mean±S.D of SGOT (U/L) in ovarian cancer patients was observed to be elevated (34.21±14.11) when compared with normal women (21.14±16.71) with p-value <0.05. Whereas, in text fig 1(G) mean±S.D of SGPT (U/L) level in ovarian cancer patients was plotted to be higher (78.22±84.98) as compared with normal persons (28.82±21.32) with significance value <0.001.







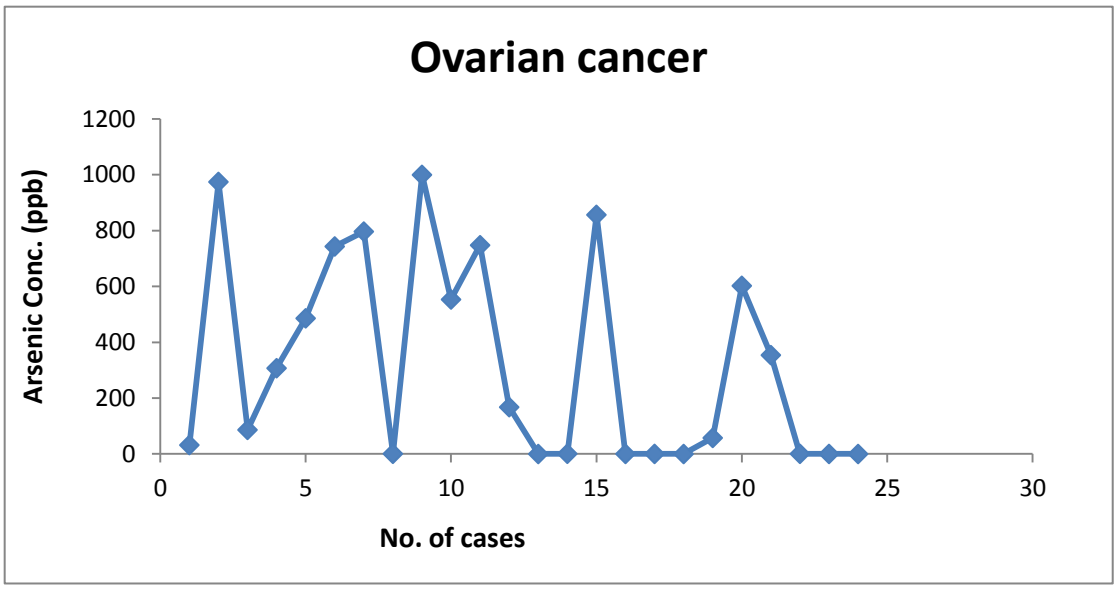


Text fig 1- Values of MDA level (A), CA-125 (B), RBC counts (C), Haemoglobin level (D), Alkaline Phosphatase level (E), Serum glutamic Oxaloacetic Transaminase (F) and Serum Glutamic Pyruvic Transaminase (G).

Table 1- Mean±S.D value of MDA level, CA-125, Haemoglobin level, RBC count, ALP, SGOT and SGPT levels with significance level of healthy women and ovarian cancer patients.

	Healthy Women mean±S.D	**Ovarian cancer cases mean±S.D**	**p-value**
MDA Level (nMol/ml)	24.39±13.39	48.87±11.97	<0.0001
CA-125 (U/ml)	31.81±9.56	133.11±293	<0.0024
Haemoglobin Level (g/dl)	13.106±1.98	7.17±3.75	<0.001
RBC count (Million cells/µl)	6.1±1.7	3.6±0.82	<0.001
ALP (U/L)	117.45±39.67	319.96±100.07	<0.005
SGOT (U/L)	21.14±16.71	34.21±14.11	<0.05
SGPT (U/L)	28.82±21.32	78.22±84.98	<0.001

Text fig. 2- Concentration of Arsenic in the blood of ovarian cancer patients.



Accumulation of arsenic in the body of ovarian cancer ranged from 0 to 1000 ppb from the data obtained from AAS. The mean±SD was calculated to be 323.739±365.994 for arsenic concentration in ovarian cancer patients. 13 patients out of 24 had extremely higher value and 9 showed negligible amount of arsenic.

Table2- Mean±SD of CA-125, MDA, RBC count, Haemoglobin, SGPT, SGOT, ALP levels for ovarian cancer patients with high arsenic and no arsenic conc.

	High arsenic (13 patients)	**Low arsenic (9 patients)**	**p-value**
CA-125	86.84±283.47	154.38±352.53	<0.0001
MDA	51.76±7.32	38.98±9.62	<0.0001
RBC count	2.4±0.58	3.9±0.76	<0.005
Hb level	6.9±3.34	7.54±3.06	<0.020
SGPT	95.41±68.73	64.03±79.54	<0.0001
SGOT	41.87±12.4	39.91±13.93	>0.05
ALP	368.77±85	309.4±98.43	<0.002

When compared, mean±SD of CA-125 was 86.84±283.47 in patients with higher and 154.38±353.53 in patients with low arsenic level with significance <0.001. MDA level was found to be increased (51.76±7.32) in high concentration as compared to patients with low or no arsenic concentration (38.98±9.62) with a very high significance. On the other hand, mean±SD of RBC count (2.4±0.58), Hb (6.9±3.34), SGPT (95.41±68.73), SGOT (41.87±12.4) and ALP (309±98.43) in patients with high arsenic level and mean±SD of RBC count (3.9±0.76

thousand/dl), Hb (7.54±3.06 g/dl), SGPT (64.03±79.54), SGOT (39.91±13.93), and ALP (309.4±98.43) in patients without arsenic level in blood with significance <0.005, <0.020, <0.0001, >0.05, <0.002 respectively.

4. DISCUSSION

Numerous environmental agents have been inscribed to cause toxicity and cancer in body. Arsenic has been indicated to be major causative agent for oxidative stress. Inorganic arsenic includes arsenite [As(III)] and arsenate [As(V)] and can be in methylated form, either as monomethylarsonic acid [MMA(V)] or dimethylearsinic acid [DMA(V)]. Both *in vitro* and *in vivo* studies of arsenic exposed animals and humans have suggested involvement of higher production of peoxyl radicals ($ROO^{\cdot}$), superoxide anion radicals ($O_2^{-\cdot}$), hydroxyl radical ($OH^{\cdot}$), hydrogen peroxide (H_2O_2), dimethylarsenic radical [$(CH_3)_2As^{\cdot}$] and non-protein sulfhydryl and oxidant induced DNA damage [11]. Oxidative stress has been found to be elevated in almost all metabolic disorders and related as one of the causative agent for cancer development. Most of the ovarian cancer is derived from epithelial cells and breast cancer of epithelial origin has been asserted to produce abnormally high MDA [12]. Increased concentration of self-generated ROS in the cells undermines the endurance of the plasma membrane resulting into apoptosis inadvertently. Such an endogenous stimulus is intensified not only in several diseases [13] and cancer but also during the toxicity caused by accumulation of pesticides [14] and arsenic. MDA level almost doubles in ovarian cancer patients when compared with healthy persons as apparent in text fig 1A. MDA is generated as the by-product of the lipid peroxidation process, acts as mutagen and possesses the capacity of activating proto-oncogenes or tumour suppressor genes leading to cancer.

Being the member of mucin family, glycoprotein, CA-125 is usually embedded in plasma membrane but due to neoplasm development in epithelial ovarian cancer, it is relieved from the cell surface by proteolytic action of certain enzyme. Concentration of MUC16 increases in peripheral blood due to which it acts as prognostic marker for ovarian cancer. According to another hypothesis, the release of CA-125 from the surface of cell may also be due to loss of fluidity of plasma membrane which abets escaping of this transmembrane glycoprotein [15]. As reflected by text fig 1B, CA-125 is too high in ovarian cancer patients as compared to healthy people which affirm CA-125 as specific and sensitive marker for early detection of ovarian cancer. Patients with higher concentration of arsenic in their body resulted with higher MDA and lower CA-125 level than patients with no arsenic level as shown in table 02.

Anaemia in cancer is quite usual which can be treated with re-oxygenation or erythropoietin doses which are indicated to provide improved survival rate in cancer [16]. Kidney begins manufacturing more erythropoietin on signal received at the time of hypoxia condition. Low level of haemoglobin and RBC count in blood signifies that erythropoietin is hypoactive which

means oxygen supply throughout the body is sufficient. Higher the oxygen-supply to the organ, greater the production of superoxides in the cell, mainly due to incomplete electron transfer or reduction of oxygen by damaged mitochondria. Oxidative stress is observed to be higher whereas RBC count and Hb level to be lower in Oca patients, indicating increased oxidative stress results in lower RBC count. Furthermore, patients with higher arsenic level show higher MDA level and lower RBC count as compared to patients with no arsenic level, which signifies arsenic and oxidative stress together have impact of RBC count and haemoglobin level. It is possible that arsenic and oxidative stress induce RBC death. In addition, arsenic has binding affinity with haemoglobin at its cys-rich area which might affect Hb level.

In addition, Alkaline Phosphatase, serum glutamic oxaloacetic transaminase and serum glutamic pryruvic transaminase are made exclusively by hepatocytes and released into blood stream. When lever secretes SGOT and SGPT when it is chafed or inflamed and releases into blood sream where it can be measured. ALP, SGOT and SGPT can be descried to be produced enormously. The reason behind this might be attributed to the toxicity level caused by several intrinsic and extrinsic factors. Several extrinsic factors like arsenite, pesticides and other toxic agents have been indicated to cause discrepancies related to livers in animal model [17]. Liver damage due to oxidative stress, apoptosis, necrosis, histological manifestations which are intrinsic factors, would intervene with normal function of hepatocytes. The increase in ALP, SGOT and SGPT in serum is due to necrosis in hepatocytes, which increases increase in permeability of the cell membrane resulting in secretion of transaminases in blood stream [18]. Liver cells are irritated by administration of several drugs, as they all have more or less negative effects on hepatocytes. SGPT, SGOT, and ALP can be analysed to be higher in the patients with higher arsenic level than patients with nil arsenic, which further aids that arsenic significantly affects the hepatocytes.

However oxidative stress is directly related to necrosis and apoptosis which manifests several malfunctions in the body but more study is needed to establish a vital relationship which would help develop better resolution for early detection of ovarian cancer.

5. CONCLUSIONS

All such parameters have been evaluated and studied extensively to establish correlation between arsenic and oxidative stress, MUC16, RBC count and Haemoglobin level and liver function tests in ovarian cancer patients. It was found that high level of arsenic increased oxidative stress in the patients which led to high MDA levels in such cases. Further, patients with high arsenic level had a relatively lower level of CA-125 as compared to ovarian cancer patients with low or no arsenic on their blood. The above findings suggest that the level of CA-125 in patients with high level of arsenic in their blood could be lower than that expected. However, this needs further corroboration from trials with large number of patients.

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