INTRODUCTION

Cervical cancer is one of the major public health problems among women worldwide and is the most common malignant neoplasms among females in many developing countries (Ferlay et al., 2008). The etiology of Cervical cancer is multifactorial. Hormonal, genetic and environmental factors appear to interplay in the pathogenesis of Cervical cancer. Also, while recent scientific advances have led to improved prevention, earlier diagnosis, and innovative treatments for many cancers, there is still tremendous unmet medical need.

Cellular antioxidant defense plays an important role in neoplastic disease to counteract oxidative damage (Looi, Mee-Lee et al., 2008). During recent decades, antioxidant Vitamins, Trace element and Minerals have received much attention in relation to cancer prevention, particularly because they may prevent free-radical damage to DNA by neutralizing free radicals and oxidants and enhance the immune system. Deficiency in some of these antioxidants, minerals and trace metals that are essential in the body may result to a number of clinical states and complications that may lead to death. Despite the findings in the diagnoses, treatment and biochemical changes in cervical and prostate cancer patients, not much work has been extensively done on the antioxidants, minerals and trace metals among cervical cancer patients. Hence, the essence to assess these antioxidants, some minerals and some trace elements in these patients.

Antioxidants

Oxidative stress has long been related to carcinogenesis in human cancers and animal cancer models. Cervical cancer is said to be mediated by Human Papilloma Virus (HPV) but recent data published also revealed role of oxidative stress in Cervical cancer. The cervical cancer patients are exposed to physical stress as well as oxidative stress which may result in the depletion of the antioxidant system and may also affect the minerals and trace metals levels essential for the body. The present study was therefore planned to understand the biochemical changes in blood of Cervical Cancer patients in order to evaluate the correlation between oxidative stress and status of Antioxidants (Vitamin A,C,E), Minerals (Ca, P, Mg) and Trace elements (Zn and Cu) in Cervix cancer. For this purpose, 25 recently diagnosed patients with squamous cells carcinoma of the cervix and 25 control subjects who had no malignancy were taken up for study. The control subjects were those who were not suffering from any cancerous lesions. The biochemical analysis of the present study supports the fact that oxidative stress in Cervical cancer patients may result in depletion of the antioxidant vitamins and vital micro and macro minerals in the body and suggest that Zinc, Copper, Magnesium and Phosphorus can be used a biomarker for the early diagnosis and possible preventive measures in the management of Cervical Cancer.
to understand its validity in diagnosis and also to follow the prognosis during the course of treatment.

**Minerals and Trace Elements**

Trace elements, in low concentrations, are integral parts of the protein structures and are essential for biological enzymatic systems to function normally. For instance, Zinc, Copper, and Magnesium are essential elements that play a vital role as cofactors for enzymes (Cobanoglu et al., 2010). Previous animal or cell-culture studies have shown that Zinc, Copper, and Magnesium are involved in the atherosogenesis process and carcinogenesis (Sadat et al., 2008) and they serve as a cofactor for the enzymes activity responsible in preventing DNA denaturation (Cunzhi et al., 2003).

Earlier studies have suggested that the Cervical Cancer patients are exposed to physical stress as well as oxidative stress. The present study was therefore planned to understand the biochemical changes in blood of Cervical Cancer patients and determine the correlation between oxidative stress by assessing the serum levels of Antioxidants (Vitamin A, C, E) and the status of Minerals (Ca, P, Mg) and Trace elements (Zn and Cu) in Cervix cancer in order to assist in the early detection of premalignant lesions and identify individuals at high risk of developing cancer. The proposed study will be therefore be highly useful for efficient management of Cervical Cancer.

**MATERIALS AND METHODS**

**Ethical approval**

Ethical approval for the study was obtained from the Institutional Ethical Committee, Erode Cancer Centre prior to the commencement of the study and the ethical principles according to the declaration were considered during the course of the research.

**Sample Collection**

25 recently diagnosed patients with squamous cells carcinoma of the Cervix and 25 control subjects who had no malignancy. The control subjects were those who were not suffering from any cancerous lesions. The female subjects within the age group of 35-65 years were selected.

**Analysis of Antioxidants**

**Sample Preparation for Analysis**

After obtaining prior consent, Venous blood was collected from the subjects under aseptic condition by vein puncture using 10ml sterile disposable syringe and needle. About 5ml of blood was collected in clot activator tube and was allowed to clot. The serum samples were stored at 4ºC before analysis and all the samples were analysed on the same day of collection. Antioxidants (Vitamins A, C, E) levels were analyzed using High performance liquid chromatography (HPLC) and LC-MS. All the results were calculated in mean.

**Estimation of Vitamin A, Vitamin C and Vitamin E**

The LC-MS technology involves use of an HPLC, wherein the individual components in a mixture are first separated followed by ionization and separation of the ions on the basis of their mass/charge ratio. The separated ions are then directed to a photo or electron multiplier tube detector, which identifies and quantifies each ion. The ion source is an important component in any MS analysis, as this basically aids in efficient generation of ions for analysis. The serum samples, were injected through an Agilent G1312B binary pump and separated by an Atlantis dC 18 column, 3 μm, 2.1 x 150 mm from Water's Corporation (Milford, 2003), using a flow rate of 0.3 ml/min and the following gradient: 100% A for 1 min, linearly increasing to 100% B from 1 to 30 min, 100% B until 40 min, and then back to 100% A at 43 min. Mobile phase A consisted of acetonitrile/ethanol/water (165/135/700 v/v/v) and mobile phase B consisted of acetonitrile/ethanol/water (539/441/20 v/v/v).

Multiple reaction monitoring (MRM) mode, product ion scanning mode and total ion scanning mode were used to analyze each compound. Negative polarity ESI was used with the following source conditions: gas temperature 325°C, gas flow 10 L/minute, nebulizer pressure 30 psi, sheath gas temperature 250°C, sheath gas flow 7 L/minute, capillary voltage 4000 V, nozzle voltage 1500V, and an electron multiplier voltage of 300V. In total ion scanning mode, the range of precursor ion mass-to-charge ratio was set between 100 and 1000. In product ion scanning mode, the mass-to-charge ratio of the precursor ion was set at 453, 479, 521 or 427 to analyze γTE-9'S, γTE-11'S, γTE-13'S or δTE-13' (2-double-bond-form, respectively). The collision energy was set at 15, 25, 35 and 45 V to observe the fragmentation pattern. The condition of gradient and ion source used in total ion scanning mode and product ion scanning mode were the same from those used in multiple reaction monitoring mode.

All data was evaluated with Agilent Mass Hunter Qualitative and Quantitative Analysis software, version B.01.04 or B.06.00

**Minerals and Trace Elements**

**Sample Preparation for Analysis**

A volume of 5ml venous blood samples were obtained by venepuncture and collected in Ethylenediamine tetraacetic acid (EDTA) bottle. Standard precautions for trace element determination were taken and samples with signs of haemolysis discarded.

Serum Zinc and Copper concentrations were estimated using direct atomic absorption spectrophotometer. The blood samples (5 ml) each were collected from each patient aseptically in clot activator tubes and were allowed to clot. The samples were then centrifuged at 2500 RPM for 10 min. The serum samples were then collected in sample vials and kept in the freezer till the samples were analysed for Calcium, Phosphorus, Magnesium. All the biochemical parameters were analysed on a fully automatic dry clinical analyser (Fujifilm-4000i). The observed results were then analysed using SPSS Software version 16.0.

**Analysis of Minerals**

**Estimation of Calcium (O-Cresolphthalein Complexone (OCPC) method)**

Calcium in alkaline medium reacts with O-Cresolphthalein Complexone to form a purple colored complex whose absorbance is proportional to the Calcium concentration. Interference due to magnesium and iron is eliminated by using

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RESULTS AND DISCUSSION

Antioxidant

Vitamin A

Vitamin A is important for healthy mucous membranes. Because the cervical lining is a mucous membrane, Vitamin A may be an important factor in regulating cervical tissue as well as the immunity of the cervical microenvironment (Cai et al., 2012). In our present study, Mean serum levels of Vitamin A were significantly lower in control group than cases with cervical cancer; Levels of Vitamin A are insignificantly low with advanced stage of cancer. Previous studies have reported that both higher Vitamin A intake and serum vitamin A levels were associated with a lower risk of cervical cancer (Zhang et al., 2012). Another study evaluated whether vitamin A could lower the risk of cervical cancer and confirmed that lower vitamin A intake was strongly associated with higher cervical cancer risk (Kim et al., 2010). Inadequate dietary intake of Vitamin A as well as action of antioxidant vitamin A on free radicals released in cervical cancer patient could be associated with decreased serum levels of Vitamin A.

Vitamin C

Vitamin C is a water-soluble antioxidant vitamin. Its role as an antioxidant is indicated by its known free radical scavenging action. As a reducing agent and antioxidant agent it directly reacts with superoxide and hydro peroxides. Decreased levels of antioxidants indicate upsurged lipid per oxidation which is a consequence of increased free radical generation of free radicals. The probable reason for the decrease in levels of Vitamin C may be associated with its action as antioxidant where it gets utilized in the role of sparing Vitamin-E, which is another lipid soluble antioxidant.

The results in fig 1 (b) showed a significant decrease in vitamin C level in serum of cervical cancer females when compared with healthy females. Similar results showed that the vitamin C concentrations decreased in the serum of patients with Cervical Cancer (Manju et al., 2010). From the studies it is clear that the increased number of free radicals may play a role in the development of cancer and other health conditions (Aghajanpour et al., 2017). So there will be a more burden on antioxidant vitamins to scavenge these extra free radicals due to cancer and may cause its depletion in circulation. Vitamin C reduces the tissues damage and removes free radicals by directly reacting with O2 - and OH-. Thus, the decrease in the Vitamin C levels may be associated with increased utilization of antioxidants to combat oxidative stress which can promote the expansion of tumors (Harris et al., 2015). Further, Vitamin C helps in reformation of Vitamin E from tocopherol radical formed as a result of lipid peroxidation and therefore Vitamin C contributes with vitamin E in protecting cells from damage (Stahl, 1997).

While Vitamin C has been proven to benefit the immune system during acute infections, there are now many studies to support the findings that vitamin C may also contribute to more effective chemotherapy and lessened damage on the body as a result of chemotherapy.

Vitamin E

Vitamin E is the major antioxidant in cell membranes where it protects membrane structures from lipid peroxidation and severe oxidative damage, which tends to be initiated at polyunsaturated fatty acids (Gillham et al., 2000). Fig 1 (c) showed a significant decrease in Vitamin E level in serum of cervical cancer females when compared with healthy females. The obtained results were in agreement with earlier studies (Manju et al., 2002; Bhuvarahamurthy et al., 1996) which showed a decrease in vitamin E level in cervical cancer patients. The reasons for the decrease in the vitamin E concentrations in cervical cancer patients might be attributed to its ability to scavenge lipid peroxides and prevent the cellular damage by the free radicals (Manju et al., 2002). Recent clinical trials have demonstrated the significant cancer preventive potential of Vitamin E in many different cancer.
sites, ranging from oral and pharyngeal cancer to prostate cancer (Shklar et al., 2000).

**Table 1** Vitamin levels in serum of cervical cancer patients

<table>
<thead>
<tr>
<th>Antioxidant</th>
<th>Control (n=60)*</th>
<th>Cervical Cancer Patients (n=60)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>VITAMIN A (µg/dl)</td>
<td>40±3.4</td>
<td>26.22±2.54</td>
</tr>
<tr>
<td>VITAMIN C (µg/dl)</td>
<td>1.3±6.4</td>
<td>0.78±3.56</td>
</tr>
<tr>
<td>VITAMIN E (µg/dl)</td>
<td>11.25±1.05</td>
<td>5.56±0.76</td>
</tr>
</tbody>
</table>

Each value is the Mean ±SD  
*Values in the parentheses indicate the number of Subjects

The results of several studies suggest that the intake of Vitamins, including Vitamin C and Vitamin E from foods or supplements, may reduce the risk of endometrial cancer (Bandera et al., 2009). Low levels of antioxidant vitamins were observed among all the clinically and histopathologically classified cases of cervical cancer patients. Deficiency of Vitamin C and Vitamin E are associated with an increase in cervical cancer stages and adverse effects of these micronutrients deficiency on cell differentiation and growth may potentate the effects of a local carcinogen or allow more rapid proliferation of malignant cells.

**Minerals**

The role of Minerals and Trace elements in cancer has been the subject of conjecture, and reports of different Researchers are often conflicting and contradictory. A major reason for these discrepancies is the difficulty in analyzing trace elements and the problems that exist in collecting specimens without contamination (Thiers, 1957). These elements are required in small concentrations as essential components of biological enzyme systems or of structural portions of biologically active constituents. In the present study, the serum levels of Calcium, Phosphorus and Magnesium in Cervical Cancer Patients were evaluated.

**Calcium**

Calcium (Ca) is an essential element for humans, available only through dietary sources. It is largely concentrated in the skeleton as calcium-phosphate complexes, serving to maintain bone strength and Calcium homeostasis. Non-skeletal Calcium which accounts for approximately one percent of the total is responsible for a wide range of biological activities, including cell signalling, muscle contraction and nerve impulse transmission (Peacock, 2010).

Serum levels of calcium and phosphorous were evaluated in patients with carcinoma of the cervix between pre- and post-menopausal women and compared the same with various normal female populations of different physiological status. No definite relationship was observed between serum calcium and phosphorous (inorganic) level. It has long been known that high calcium levels in the blood are quite common in people with cancer, particularly late stage cancer. This is because in late stage cancer the cancer has spread to bone or the cancer secretes substances that cause calcium regulation within the body to be faulty. Hence it would be expected to notice Hyperkalaemia in patients with Cervical Cancer, but all the patients in our study were Normocalcemic. Cho and colleagues (Cho et al., 1991) suggested two possible explanations for these results. First, calcium reflux from bone may have been too subtle to be detected. Another explanation is that some cases of malignancy may have been associated with elevated levels of bone-resorbing material even in the absence of Hypercalcemia because of regulatory mechanisms that maintain Normocalcemia. (Henderson et al., 1990).

**Phosphorus**

Phosphorus is an important element that is vital to several of the body’s physiological processes. It helps with bone growth, energy storage, and nerve and muscle production. It has been already reported, few decades ago, that phosphorus increases in the blood in various conditions and especially in cancer due to
the increased requirements of the rapidly growing cells. Our study also revealed an increase in Phosphorus level in serum of Cervical Cancer Patients compared with the control.

**Magnesium**

Magnesium (Mg) is the second most abundant intracellular cation in the body, involved with numerous biological activities particularly related to its interaction with Calcium. Levels of both these cations are regulated through competition for intestinal absorption and renal reabsorption and also via a negative feedback system (Dai, 2011). It has been indicated that the physiologic effects of Ca are enhanced in Mg deficiency as they compete for intracellular membrane binding sites (Iseri, 1984). Our study found a reduction in mean serum level of Magnesium among participants with histologically diagnosed Cervical Cancer. Magnesium deficiency may increase or decrease the risk of carcinogenesis paradoxically, too. Previous studies on the serum level of magnesium and manganese are scarce with paradoxical results. Some reported a significantly lower serum level of magnesium and higher serum level of manganese in patients with cancer compared with the control group.

**Trace Elements**

**Zinc**

In this study, we investigated the levels of serum trace elements in patients diagnosed with Cervical Cancer compared to Cancer-Free controls. The findings from our present study indicated a strong association of low mean serum levels of Zinc with invasive squamous cells carcinoma of the cervix. Previous studies have also reported that serum Zinc concentrations were decreased in patients with Ovarian, Testicular, Cervical, Bladder and Renal Cancer. Zinc plays an anti-carcinogenic role through structural stabilization of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and ribosome. It has a protective effect against free-radical injury. Zinc also has an important role in transcription, antioxidation, and DNA repair. Therefore, zinc deficiency may be involved in DNA breakdown and as a result, oxidative changes may increase the risk of cancer. Some authors have even proposed that serum level of Zinc can be considered as a marker for evaluating the prognosis of Cancer. Hence, due to decline in the concentration of Zn in Cervical Cancer patients any of the above process could get disturbed and may be acting as a causative agent for cancer.

**Copper**

Copper (Cu) modulates the activities of multiple enzymes, regulates the redox state, promotes angiogenesis and mediates cellular proliferation. As a result of these activities, Cu appears to play an important role in the carcinogenic process which is evident through the increase seen in Cu levels in cancerous tissues (Majumder et al., 2009).

In our study, the mean serum concentration of Copper in the cancer patients was lower than in the control. In contrast there are paradoxical results regarding the Copper level in cancer cases. Some researchers reported higher serum level of Copper in patients with cancer compared with the control group. But other studies showed lower serum level of copper in patients with cancer compared with the healthy controls.

Copper plays a role in the production of haemoglobin, myelin, collagen and melanin as an essential nutrient (Adelstein,1961) and studies have shown that normal immune function requires adequate Cu intake. Copper is also essential in antioxidant defense, but higher concentrations of copper is associated with neurodegenerative disorders through induction of cellular

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**Table 2 Concentration of Minerals in Serum of Cervical Cancer Patients**

<table>
<thead>
<tr>
<th>Minerals</th>
<th>Control (n=25)*</th>
<th>Cervical Cancer Patients (n=25)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (mg/dl)</td>
<td>9.2±0.84</td>
<td>9.29±0.78</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>3.5±0.26</td>
<td>4.24±0.35</td>
</tr>
<tr>
<td>Magnesium (mg/dl)</td>
<td>2.1±0.15</td>
<td>1.46±0.12</td>
</tr>
</tbody>
</table>

Each value is the Mean ±SD

*Values in the parantheses indicate the number of Subjects

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![Fig 2 (a) Serum Calcium levels in Cervical Cancer Patients](image1)

![Fig 2 (b) Serum Phosphorus levels in Cervical Cancer Patients](image2)

![Fig 2 (c) Serum Magnesium levels in Serum of Cervical Cancer Patients](image3)
overgrowth and eventually result in free hydroxide radical-DNA damage. On the contrary, Copper deficiency may lead to an increase in oxidative damage.

### Table 3 concentration of trace elements in serum of cervical cancer patients

<table>
<thead>
<tr>
<th>Trace Elements</th>
<th>Control (n=25)*</th>
<th>Cervical Cancer Patients (n=25)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zinc (µg/dl)</td>
<td>88±26.4</td>
<td>67.22± 8.35</td>
</tr>
<tr>
<td>Copper (mg/dl)</td>
<td>85± 5.8</td>
<td>71.0± 5.2</td>
</tr>
</tbody>
</table>

Each value is the Mean ±SD
*Values in the parantheses indicate the number of Subjects

From the previous studies it is apparent that Electrolyte abnormalities are common and recognizable features in cancer patients and some studies attributed electrolyte abnormalities with drinking demineralized tap water. Electrolyte disorders in patients with cancer can be secondary to either the cancer or its therapy. Hence more studies need to confirm the possibility of using these elements as the diagnosis or prognosis tool of the Cervical Cancer disease.

**SUMMARY AND CONCLUSION**

In our present study, the status of oxidative damage was investigated by measuring the antioxidants Vitamin A, Vitamin C and Vitamin E in Cervical cancer patients.

The results of the present study revealed a reduction in all serum antioxidant nutrients levels, Vitamin A, Vitamin C and Vitamin E in women with cervical cancer. This study therefore clearly suggest the scavenging activity of antioxidants against the free radicals in the pathogenesis of cervical cancer and provides some evidence for the protective role of the serum concentration of antioxidant Vitamins (Vitamin A, Vitamin E, and Vitamin C) in the etiology of cervical cancer. Also Coexisting deficiency of antioxidants may potentially increase the risk for cervical cancer and the implication in terms of prevention is to encourage intake of fresh vegetables and fruits rich in antioxidant vitamins. Replication of this study in different populations may give further credence to its findings.

Minerals and Trace elements have an important role within human body such as protection against cellular oxidative stress, production of proteins and synthesis and structural stabilization of nucleic acids. Over recent years many studies done if these elements have any effect in the etiology of neoplastic disease and in alteration of antioxidants levels. From the results of our present study, it is observed that the Zinc and Copper levels are found to be significantly lower in Cervical Cancer patients compared to control. Similarly Serum Zinc level is also decreased in Cervical Cancer patients. However mean Serum Phosphorus level is found to be higher than the Control and Calcium level was well within the normal range. Trace elements have an important role in biological processes as they consider as cofactors for many enzymes, included antioxidant enzymes. A deficiency of these trace elements can influence the level of oxidative stress causing increase of free radicals and increased their adverse effect on cell proliferation and direct effects on DNA, leading to genetic mutations or genomic damage. Hence the results of the biochemical analysis of Cervical Cancer suggest that Zinc, Copper, Magnesium and Phosphorus can be used a biomarker for the early diagnosis and possible preventive measures in the management of Cervical Cancer. However, future robust prospective studies are needed to determine if these trace element concentrations will impact clinical outcomes and also to establish whether routine provision of these trace elements as supplements, will result in improved Cervical Cancer treatment outcomes, in Cervical Cancer affected women.

**Future Prospects**

Cancer is caused by culmination of environmental and genetic factors, characterised by a series of genetic and epigenetic changes, manifested as molecular alterations occurring at various stages in the development and progression of the disease. The outcome of the present study clearly hint that patients with Cervical Cancer are at risk for electrolyte imbalances from a myriad of causes, and continuous assessment of their status is often necessary. Hence in future, it is recommended to consider the trace elements from the standpoint of both their role in carcinogenesis and the possible use of their assay in biological fluids as diagnostic or prognostic aids in patients with cancer.

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Conflict of Interest
The Author has no conflict of interest.

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