Disparities in Characteristics, Symptoms and mtDNA Influence on Ovarian Cancer Patients From South Indian Population

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Abstract

Ovarian cancer (OC) is a complex multifactorial malignancy, ranking as second most common reproductive cancer in India. The purpose of this study was to compare whether different characteristics combined with hormonal and genetic influence among the urban, rural and tribal populations affected with OC would have a significant impact on OC incidence. To address this challenge, we designed a statistical approach that combines individual-level epidemiological data from both OC patients and controls from designed study populations using questionnaires. A total of (n=72) OC patients were obtained from several oncological clinics, hospitals and population-based surveys from various districts of South Indian population. Using validated hormonal biomarkers, we estimated hormonal profiles in both OC subjects and controls to measure the role of hormones in OC severity and incidence. Further we conducted mitochondrial gene sequencing, we found 5 nucleotide changes among which MT9140 C>T) was the predominant in OC subjects. In conclusion, this kind of study would be the first of its kind, where population-based epidemiological surveys serve as keys for comparing the characteristic factors and genetic influence associated with OC incidence. Hence, we strongly recommend that frequent conduction of epidemiological studies and health awareness camps would be beneficial to overcome such lethal conditions.

Introduction:

Ovarian cancer (OC) is the second most common lethal feminine cancer especially among women within 50–69 years of age. It is a malignant form of tumor associated with spread of cancer in stroma, distant metastasis, chemoresistance, angiogenesis and disease relapse. Growing evidences suggests that, the incidence of OC differs among various populations based on their residence, lifestyle factors, socio-economic and educational status. It is also stipulated that different lifestyle factors have been connected with the delayed diagnosis of OC. The disease remains asymptomatic in the early stages and thus mostly diagnosed in the advanced metastatic stage, which makes this cancer a deadly one. Early screening of various risk factors may be an effective technique to treat and decrease the mortality rate of OC patients. The use of oral contraceptive pills and in vitro fertilization (IVF) mode of pregnancy has emerged as a prime agent for pathogenesis of OC. Menarcheal age another major risk factor for OC, was found to be directly linked with production of ovum in women.

There is an immediate urge to develop novel strategies to understand the mechanism behind the pathogenesis of OC. Recent reviews have identified some novel alterations in mtDNA and also interpreted that mutated mtDNA transmission via maternal lineages in OC. Until now, the major gene focused in OC is BRCA1/2. Mutational analysis, such as the sequencing of a specific gene of interest, has greatly facilitated in identifying the mutations in crucial oncogenes and tumour suppressors genes causing OC. However, in a recent study, it was reviewed that Sp1 a major transcription factor plays a pivotal role in OC, as it controls the expression of pro-oncogenes which takes part in progression of OC. The shift of research from nuclear gene to mitochondrial DNA (mtDNA) gene is due to its high rate of mutations mainly because of the production of reactive oxygen species (ROS) during OXPHOS. These studies suggest that women from different regions, socio-economic, age and ethnic groups show variation in symptoms and alterations in genomic frequencies that contribute to OC. Thus, study based on epidemiology and characteristics in OC patients would emerge as a promising tool for early diagnosis and effective treatment.

India is a country with diverse populations living with varied backgrounds, religion and ethnicities. In populations from different regions like urban, rural and tribal areas the exposure and knowledge regarding OC varies drastically. Very few reports suggested that OC patients living in tribal and rural areas were more prone to advanced cancer stage due to late diagnosis, when compared to the urban population. Further, in OC, the primary hormones that get elevated are luteinizing hormone (LH), follicle-stimulating hormone (FSH) and estrogen. All these three hormones have been produced by the ovaries. Estrogen has long been suspected as one of the major etiologic factors of OC. Thus, the aim of the present study was to compare the differences in characteristics and symptoms of the OC patients living in urban, rural and tribal areas from South Indian population. Additionally, we have also analysed the levels of hormones such as estradiol, LH and FSH and mtDNA influence among the study subjects. Hence, this study would be a pathbreaker for women with OC, to help them overcome their problems by analysing the characteristic symptoms and genetic influence in the early stages of the disease.

Result:

In the present study, 72 women diagnosed with OC who met the study inclusion criteria were recruited. The subjects were mainly categorized based on three different populations such as urban (n = 46), rural (n = 18) and tribal (n = 08). OC patients were grouped based on their age, as group I (≥ 55) and group II (≥ 55), in which the group I urban population, comprised of 18 (39.1%) subjects and 28 (60.8%) were of group II subjects. In rural population, group I consisted of 6 (33.3%) subjects and 12 (66.6%) subjects were in group II. Finally, tribal population group I consisted of 3 (37.5%) subjects and 5 (62.5%) subjects were in group II.

Table 1 shows, the general characteristics (educational qualification, food habit, and parity status) of OC patients and their controls. With regard to literacy rate of group I, the data revealed that the incidence of OC was more in the urban population (20.8%) followed by rural (2.77%) and tribal (1.38%) population when compared to their respective control subjects [urban-25%, rural-5.55% and tribal-2.77%]. While the literacy rate of group II data revealed that the incidence of OC was more in the urban population (25%) followed by rural (4.16%) and tribal population (0%) when compared to their respective control subjects [urban-23.6%, rural-8.33% and tribal-4.16%]. The data pertaining to illiteracy rate of group II revealed that the prevalence of OC was more in tribal population (6.94%) followed by rural (12.5%) and urban (13.8%) population when compared to their respective control subjects [tribal-2.77%, rural-8.33% and urban-0]. A similar trend was observed for the illiteracy rate among the group I subjects of the present study. Further for the food habits, the non-vegetarian diet intake was found to be associated with a greater risk for OC among the group I and group II subjects. More cases among the group I subjects from urban (19.4%), followed by rural (6.94%) and then the tribal (4.16%) were consuming non-vegetarian diet on daily basis, when compared to the control subjects [urban (5.55%), rural (2.77%) and tribal (1.38%) respectively. Similarly, the data of non-vegetarian diet in the group II subjects showed that more subjects were prone to get OC in urban (30.5%) areas, than the rural (11.1%) and the tribal (6.94%) areas when compared to their control subjects [urban (13.8%), rural...
(5.55%) and tribal (1.38%) respectively. Thus, a fivefold increase of OC was observed among the group II OC patients due to their high consumption of non-vegetarian food daily. Among the study population, the data on parity status showed that the women with OC from urban populations of both group I (8.33%) and group II (8.33%) showed higher rate of null-parity (no children) when compared to their control subjects [urban group I (2.77%) and group II (1.38%)] respectively.

| Table 1 | Comparison of General Characteristics between OC and Control Subjects |
|---------|---------------------------------------------------------------|
|          | Group I (<55) | Group II (≥55) |
|          | Urban | Rural | Tribal | Urban | Rural | Tribal |
| Educational Qualification |       |       |       |       |       |       |
| Literacy rate of OC | 15 (20.8%) | 2 (2.77%) | 1 (1.38%) | 18 (25%) | 3 (4.16%) | 0 (4.16%) |
| Literacy rate of Control | 18 (25%) | 4 (5.55%) | 02 (2.77%) | 17 (23.6%) | 06 (8.33%) | 03 (8.33%) |
| Illiteracy rate of OC | 3 (4.16%) | 4 (5.55%) | 2 (2.77%) | 10 (13.8%) | 9 (12.5%) | 5 (12.5%) |
| Illiteracy rate of Control | 0 (2.77%) | 02 (1.38%) | 01 (1.38%) | 11 (15.2%) | 06 (8.33%) | 02 (8.33%) |
| Food Habits |       |       |       |       |       |       |
| Veg in OC | 4 (5.55%) | 1 (1.38%) | 0 (8.33%) | 6 (8.33%) | 4 (5.55%) | 0 (5.55%) |
| Veg in Control | 14 (19.4%) | 04 (5.55%) | 02 (2.77%) | 18 (25%) | 08 (11.1%) | 04 (11.1%) |
| Non-Veg in OC | 14 (19.4%) | 5 (6.94%) | 3 (4.16%) | 22 (30.5%) | 8 (11.1%) | 5 (6.94%) |
| Non-Veg in Control | 4 (5.55%) | 02 (2.77%) | 01 (1.38%) | 10 (15.2%) | 4 (8.33%) | 01 (8.33%) |
| Parity Status |       |       |       |       |       |       |
| Nulliparity in OC | 6 (8.33%) | 1 (1.38%) | 0 (8.33%) | 6 (8.33%) | 3 (4.16%) | 1 (1.38%) |
| Nulliparity in Control | 2 (2.77%) | 0 (1.38%) | 0 (2.77%) | 1 (2.77%) | 2 (1.38%) | 0 (1.38%) |
| Para-parity in OC | 12 (16.6%) | 5 (6.94%) | 3 (4.16%) | 22 (30.5%) | 9 (12.5%) | 4 (5.55%) |
| Para-parity in Control | 16 (22.2%) | 6 (8.33%) | 3 (4.16%) | 27 (37.5%) | 10 (13.8%) | 5 (6.94%) |

OC – Ovarian cancer; Veg – Vegetarian; Non-Veg – Non-Vegetarian.

Table 2 shows the mean, standard deviation, confidence interval and p – value among the characteristics in the different populations. Age at menarche was not found be associated with the incidence of OC among the tribal populations belonging to both (group I and group II) (n = 03). A similar trend was observed in the rural (n = 11) subjects. With regard to urban subjects a significant increase in the incidence of OC was observed in both group I and group II (n = 17) (p = 0.0001). With respect to medication, the tribal population belonging to both (group I and II) who consumed natural medicines without any prescription showed less prevalence for OC (n = 5), followed by the rural (n = 7) population. Whereas the urban subjects belonging to group I and group II showed high incidence of OC (n = 17) (p = 0.0001). A higher number of women who had undergone IVF treatment showed incidence of OC among urban (n = 11) subjects when compared to that of rural (n = 0) and tribal (n = 0) (p = 0.12) women. Using the circos plot we have depicted the results elaborately (Fig. 1).
Table 2
Reproductive characteristics between OC and Control subjects

| Variables | Urban | Rural | Tribal | Mean ± SD | OR | CI (95%) | P-value |
|-----------|-------|-------|--------|-----------|----|----------|---------|
| GI        | GII   | GI    | GII    | GI        | GII|          |         |
| Menarche  | 6     | 12    | 5      | 6         | 1  | 2        | 5.33 ± 3.54 | 10.72 | 3.8630–29.748 | 0.0001* |
| OC        | 2     | 4     | 1      | 2         | 0  | 1        | 1.66 ± 1.24 |
| Medication| 4     | 13    | 3      | 4         | 2  | 3        | 4.83 ± 3.71 | 9.03  | 3.2475–25.148 | 0.0001* |
| OC        | 1     | 5     | 1      | 1         | 0  | 1        | 1.5 ± 1.6   |
| IVF       | 6     | 5     | 0      | 0         | 0  | 0        | 1.83 ± 2.6  | 2.41  | 0.794–7.3515  | 0.12   |
| OC        | 2     | 1     | 0      | 0         | 0  | 0        | 0.5 ± 0.76  |

OC – Ovarian cancer; IVF – in vitro fertilization; OR – odds ratio; CI - confidence interval

Table 3, Figs. 2 and 3 shows a comparative analysis of the symptoms for OC among the three populations. In our study, the data showed that the most commonly associated symptom for OC in all the three populations was frequent urination n = 26 (36.11%). For group I subjects it showed that in urban areas (15.2%) of them had symptoms of frequent urination followed by (11.1%) rural and (12.5%) tribal population. While in the group II subjects (19.5%) of the urban population had frequent urination, (22.2%) of the rural and (37.5%) of the tribal population had the same respectively. The incidence of the least common symptom observed within these populations was indigestion n = 6 (8.33%). For group I subjects, it showed (0%) of urban, (5.5%) of rural and (12.5%) of tribal women had this symptom. While group II subjects had shown (4.34%) urban, (11.1%) rural and (0%) tribal symptoms for indigestion.

Table 3
Symptoms associated with OC subjects

| Population Groups | Symptoms | Abdominal or pelvic pain | Sudden weight loss | Frequent urination | Indigestion | Abdominal bloating |
|-------------------|----------|--------------------------|-------------------|-------------------|-------------|-------------------|
| Urban Group I     | 6(13.0%) | 4(8.69%)                 | 7(15.2%)          | 0                 | 2(4.34%)    |
| Group II          | 7(15.2%) | 5(10.8%)                 | 9(19.5%)          | 2(4.34%)          | 4(8.69%)    |
| Rural Group I     | 1(5.55%) | 1(5.55%)                 | 2(11.1%)          | 1(5.55%)          | 2(11.1%)    |
| Group II          | 2(11.1%) | 1(5.55%)                 | 4(22.2%)          | 2(11.1%)          | 2(11.1%)    |
| Tribal Group I    | 0        | 0                        | 1(12.5%)          | 1(12.5%)          | 0           |
| Group II          | 1(12.5%) | 0                        | 3(37.5%)          | 0                 | 2(25%)      |
| Total             | 17       | 11                       | 26                | 06                | 12          |

The Table 4 depicts the LH, FSH and estradiol hormone levels in OC patients and their control subjects. The OC patients showed significantly high levels of LH in all the three populations, viz., urban (p = 0.0002); rural (p = 0.001) and tribal (p = 0.002) for LH hormones. Likewise, the estradiol hormone levels were high in all the three populations when compared to their respective controls (p = 0.0001). Further, it was found that the FSH levels were low in OC patients in all the three populations viz., urban (p = 0.02); rural (p = 0.01) and tribal (p = 0.01) compared to their controls. Further Fig. 4 is a pictorial representation of the forest plot where it is observed that all the three hormone levels are significantly associated in causing OC among the study populations compared to their controls respectively.
mean that they encounter OC less than the people living in urban areas residing in urban areas with OC living in rural or tribal areas are more prone to the disease due to late diagnosis and insufficient hospital care, when compared with population of disease and access to care such as age, sex, race, socio-economic status, past medical history, stages of the disease, knowledge about the symptoms of disease, facilities for diagnosis the overall survival rates of various populations within a country. These population-based studies, can compare the differences between the characteristics (NCDIR) of Indian Council of Medical Research (ICMR). Although epidemiological surveys are not reported in large numbers, it is the best tool to understand in various urban, rural and tribal populations based on the registries operating under the network of National Center for Disease Information and Research with metastatic stage of OC is approximately 27.3% for 5 years. In India, during the period 2015–2020, occurrence of OC has increased from 45231–59276, Ovarian cancer (OC) is a complex condition that occupies the fth spot as the cause of mortality in all gynaecological cancers. The prevalence rate of OC is

Discussion:

In the gene encoding the subunit 6 of ATPase (MTATP6), we identied 5 nucleotide changes among our 72 OC subjects from urban, rural and tribal population. One heteroplasmic variation (MT9140 C > T) was found in 51.1% of the OC subjects. Among these mutations 2 were synonymous and 3 were missense mutation which has been elaborately explained (Table 5). Although epidemiological surveys are not reported in large numbers, it is the best tool to understand in various urban, rural and tribal populations based on the registries operating under the network of National Center for Disease Information and Research with metastatic stage of OC is approximately 27.3% for 5 years. In India, during the period 2015–2020, occurrence of OC has increased from 45231–59276, Ovarian cancer (OC) is a complex condition that occupies the fth spot as the cause of mortality in all gynaecological cancers. The prevalence rate of OC is

Table 4

Hormonal levels associated with OC and Control subjects

| Variables | Urban | OR | Cl | P-value | Rural | OR | Cl | P-value | Tribal | OR | Cl | P-value |
|-----------|-------|----|----|---------|-------|----|----|---------|--------|----|----|---------|
| LH        | 25.8± | 44.2± | 0.20 | 0.09- | 0.0002* | 26.2± | 42.9± | 0.28 | 0.12- | 0.001* | 23.0± | 40.8± | 0.28 | 0.12- | 0.002* |
| LH        | 12.5± | 33.2± | 3.55 | 4.03   | 0.57  | 1.89 |
| FSH       | 7.77± | 13.3± | 0.28 | 0.08- | 0.02* | 7.75± | 13.0± | 0.24 | 0.07- | 0.01* | 7.37± | 13.4± | 0.24 | 2 | 0.01* |
| FSH       | 13.2± | 25.6± | 1.51 | 2.04   | 0.42  | 1.99 |
| OC        | 81.8± | 7.84± | 39.1 | 1.40   | 8.68± | 8.46± | 0.021 |
| Estradiol | 173±  | 17.2± | 0.008 | 0.003- | 0.0001* | 169±  | 16.3± | 0.009 | 0.003- | 0.0001* | 175±  | 17.4± | 0.009 | 0.003- | 0.0001* |

P-value is significant less than 0.05 OR – Odds Ratio; Cl – Con dent interval; Syn – Synonymous; Non-Syn – Non-Synonymous; I – Isoleucine; A – Alanine; V – Valine; L – Leucine; T – Threonine

Table 5

Polymorphism and Mutation in MT-ATP6 gene in OC patients

| Gene Name | Nomenclature of Mutation | Frequency of Mutation | Syn/ Non Syn | Amino Acid Change | OC subject | OR | Cl | P-value |
|-----------|--------------------------|-----------------------|--------------|------------------|------------|----|----|---------|
| MT-ATP6   | 8598 T > C               | 20.8                  | Synonymous   | I                | Urban 8    | 6  | 1  | 0.26 | 0.136 to 0.507 | 0.0001* |
|           | 9140 C > T               | 51.1                  | Missense Mutation | A > V | 37 | 10 | 3 | 2.27 | 1.249 to 4.134 | 0.007* |
|           | 8541 G > A               | 34.7                  | Synonymous   | L                | 11  | 11 | 0  | 0.44 | 0.241 to 0.800 | 0.007* |
|           | 8860 A > G               | 45.8                  | Missense Mutation | T > A | 19 | 9  | 5  | 0.84 | 0.479 to 1.491 | 0.005* |
|           | 9118 C > G               | 37.5                  | Missense mutation | L > V | 14 | 9  | 4  | 0.63 | 0.357 to 1.131 | 0.01* |

Discussion:

Ovarian cancer (OC) is a complex condition that occupies the fifth spot as the cause of mortality in all gynaecological cancers. The prevalence rate of OC is almost 10–15 per 100,000 women throughout the world and occurs as a major problem among women. The overall survival rate for women diagnosed with metastatic stage of OC is approximately 27.3% for 5 years. In India, during the period 2015–2020, occurrence of OC has increased from 45231–59276, in various urban, rural and tribal populations based on the registries operating under the network of National Center for Disease Information and Research. Although epidemiological surveys are not reported in large numbers, it is the best tool to understand the overall survival rates of various populations within a country. These population-based studies, can compare the differences between the characteristics such as age, sex, race, socio-economic status, past medical history, stages of the disease, knowledge about the symptoms of disease, facilities for diagnosis of disease and access to care among the urban, rural and tribal population of a particular region. Very few reports have mentioned that patients diagnosed with OC living in rural or tribal areas are more prone to the disease due to late diagnosis and insufficient hospital care, when compared with population residing in urban areas. While some studies have contradictory reports suggesting that the lifestyle pattern followed by the rural and tribal people would mean that they encounter OC less than the people living in urban areas. Therefore, it can be predicted that people living in different regions or locations...
has an effect on the outcome of the OC especially due to the lifestyle factors, socio-economic status, educational qualification and access to healthcare facilities in urban, rural and tribal areas.

The present study, showed that the lifestyle factors such as educational qualification, food habit and parity status have a significant influence on the prevalence of the OC disease among the study subjects. Similar results were reported in several studies stating that education plays a vital role in the maintenance and prevention of the OC. The study accompanied the findings of Thigpen et al. where the women belonging to group II (≥55 age) were more affected with OC when compared to the group I (<55 age). Thus, it can be concluded that several lifestyle factors such as literacy, food habits, parity status and age would be risk factor for incidence of OC in women.

The age wise distribution was analyzed in maximum women living in urban area experience menarche at an age group of 15–18, when compared to women from rural and tribal who attain menarche by 11–13. This is in accordance with a previous study, where the menarcheal age was directly associated with OC risk. There has been substantial evidence that early menarche and late menopause increases the risk and may be seen as causes of OC. The association between menstrual history and the risk of epithelial OC has been studied frequently. Further, we observed that, the consumption of oral contraceptive pills was more common among the women living in urban areas when compared to the rural and tribal areas. Similarly, La Vecchia and colleagues had reported that favourable trends of OC in most of the metropolitan cities due to overconsumption of medications/oral contraceptive pills in the cohorts born after 1930. On contrary, another study reported that use of oral contraceptives is infrequent and there were no variances among the place of residence in the study subjects. In our study, we found that only women living in urban regions undergo IVF techniques for pregnancy; hence this parameter is not a significant risk factor for OC in our subjects. This was in agreement with an earlier study, where women following IVF had no evidence for increased risk of OC.

The most frequent symptoms observed in OC patients were abdominal or pelvic pain, sudden weight loss, frequent urination, indigestion and abdominal bloating. Our study indicates that among the common symptoms of OC, most frequently observed symptom in all the three populations is frequent urination while the least one is indigestion. On contrary, another study showed that women with OC showed more prevalence for symptoms associated with abdominal distension. Thus, positive prediction values about the symptoms of OC can be a realistic suggestion for accelerating the diagnosis of OC in early identification of symptoms associated to this disease.

In reproductive related gynaecological cancers in women, hormones do play a pivotal role as a causative factor for its prognosis and progression. Among these, estrogen, LH and FSH hormones, can be used as a marker to detect OC in the early stage. It has been reported that the GnRH hormones are produced in the healthy granulosa cells of the ovary. Earlier it has been suggested that in patients who are suffering from OC, there is an elevation in the levels of LH when compared to the healthy patients mainly due to the mutations which occur in the LH receptors. Similarly, we found that the OC patients showed significantly higher levels of LH when compared to the normal levels among their respective controls. A case-control study of 88 post-menopausal patients, revealed that women who are having low-levels of FSH the circulating hormone, were at a higher possible risk of developing OC in their lifetime. McSorley et al. carried out a nested case-control, where the authors found that the OC patients with higher levels of circulating FSH showed a decreased risk of acquiring OC in their lifetime. The present study was in accordance with the previous findings, where it showed that, the OC patients had significantly low levels of FSH when compared to their controls. Further, estrogen is one of the hormones which is produced and secreted inside and from the ovary. Thus, the level of estrogen hormone is almost 100-fold higher than the circulating hormone levels, whereas the estrogen present in the follicles are even higher. Earlier study reported that women who were obese, with persistent elevated levels of estrogen presented a favourable condition for the development of OC. Our study agrees with the earlier studies, where it was observed that, the OC patients showed higher level of estrogen in both the group I and II when compared to their control subjects.

The current study has been a stepping stone among the OC patients from South Indian population, where the plasma mtDNA has been utilised for diagnostic, predictive and also as a prognostic marker to detect OC in earlier stages. The MT-ATP6 appeared more susceptible to mutation than ATP8, which may reflect changes in energy metabolism among cancer cells. In an earlier finding, the authors found that G > A replacement in the MT-ATP6 gene among the breast cancer patients, because of the exposure to the free radicals. Even though the sample size of different groups is limited, the present study will be an evident in consistent with the involvement of such mutations in OC. A variant 9140 C > T was observed in the MT-ATP6 gene at a frequency of 51.1%. Thus, our present findings also corroborate with the previous findings where the authors have stated that mutations in MT-ATP6 could contribute for the processes related to the either onset or progression of OC.

In conclusion, we observed that most of the OC risk factors were more common among in the patients residing in urban population when compared to the rural and tribal populations. Also, this study has disclosed that hormonal studies would help to detect OC patients in the early stages of their life, which will enable us to treat the disease conditions appropriately. To sum up, this population – based epidemiological survey is an indicator of the need to identify the target populations in varied regions and to provide educational interferences about the environmental and health – care support to help the patients with OC to lead a longer and healthier life. Through this study, we strongly suggest that more epidemiological surveys and targeted health awareness programs should be conducted for betterment of the life of women with OC.

**Methodology:**

**Study Site:**

The Coimbatore district lies between 11.0168°N and 76.9558°E latitude and longitude. It is one of the most attractive, beautiful and modern cities of the south region of India. Chennai district lies within 13.0827°N and 80.2707°E. It is one of the most populous cities of India and the Capital of Tamil Nadu state located in southern most part of Indian peninsula. Nilgiris district is lying in the 11.4916°N and 76.7337°E latitude and longitude. It is one of the smallest
district in the Tamil Nadu which contains a range of mountains where the climate is always cold. Dharmapuri is a town that lies in the western part of Tamil Nadu which has comes under the latitude and longitude of 12.1211°N and 78.1582°E. From these districts the samples were categorised as urban (n=46), rural (n=18) and tribal (n=08). The study data was categorized based on age as group I (<55) and group II (≥55). Based on the categorization, the urban group I consists of n=18 and group II, n=28, in rural group I, n=06 and in group II, n=12, whereas in tribes group I comprised of n=03 and group II, n=05.

**Study population:**

Totally 72 OC patients were selected from urban (n = 46), rural (n =18) and tribal (n=08) populations and the blood samples and questionnaire was obtained from various oncology clinic, medical hospitals and population-based survey from Coimbatore, Chennai, Dharmapuri and Nilgiris districts, South India. The control group was also selected according to the age matched and population residing region.

**Study design:**

Institutional Human Ethical clearance was obtained from "The Avinashilingam University for Women, Coimbatore (Ref No. AUW/IHEC-17-18/ZOO/FHP-10), and Balaji Medical College – Chennai (Ref No. 002/SBCIHEC/2017/986). The Helsinki (1966) declaration was followed through out the study. Informed consent had been obtained from the study subjects. A brief flow chart describes the study design which has been depicted in fig. 5.

**Inclusion criteria:**

The following categories were considered as the prime inclusion criteria among the OC subjects for the present study includes age, type I and II OC, stage I and II of OC, OC patients undergone chemotherapy and surgery, education qualification, parity status and lifestyle (IVF, medications and menarche).

**Exclusion criteria:**

The patients who were bed-ridden, immunological diseases, Gestational women, or any other genetic disorders were excluded from the present study. OC patients who had undergone radiation therapy were also excluded from this study.

**The survey questionnaire:**

The survey questionnaire was aimed at screening the patients with OC in three different populations including urban, rural and tribes. The survey consisted of three sections. Section 1 focused on demographic details like age, education, food habit, and parity status. Section 2 consisted of questions about specific characteristics observed in OC patients, which included the age of menarche, intake of natural or oral contraceptive medicines and pregnancy attained due to IVF. Section 3 concentrated on symptoms of OC in patients, particularly abdominal pain or pelvic pain, sudden weight loss, frequent urination, indigestion and abdominal bloating. A team of oncologist and gynaecologist had developed the questionnaire and it covered most of the common symptoms found in OC. Language experts translated the questionnaire from English to Tamil.

**Blood Sample Collection:**

Blood Sample Collection: Peripheral blood of about 10 mL was collected from the individuals through venipuncture and were stored in anticoagulants tubes. After collection, minimal amount of serum was harvested in Eppendorf tube and stored at -80°C until laboratory use.

**Hormonal Assay:**

Dispense 50 μl of standard, specimens, and controls into appropriate wells. Dispense 100 μl of Enzyme Conjugate Reagent into each well. Gently mix for 30 seconds. Incubate at room temperature (18-25°C) for 45 minutes. Dispense 100 μl TMB Reagent into each well. Gently mix for 10 seconds. Incubate at room temperature in the dark for 20 minutes. Stop the reaction by adding 100 μl of Stop Solution to each well. Gently mix for 30 seconds. Read the optical density at 450 nm with a microtiter plate reader within 15 minutes. The protocol was conducted using the Abcam hormonal kit and the protocol was followed according to the manufacturer's instruction.

**MT-DNA isolation from blood:**

The mtDNA protocol was conducted according to the Thapa et al., The postnuclear supernatant fraction was collected in the 30 mL centrifuge tube and centrifuged at 15000 g at 4°C for 30 min to sediment the mitochondrial pellet. Wash the pellet thrice with low salt buffer (TKM1), to ensure complete elimination of lysed erythrocytes. Then the pellet was transferred to a 2 mL Eppendorf tube and suspended in 480 μl of high salt buffer TKM2followed by addition of 75 μl of 10% SDS. Then it was incubated at 55°C in the heating block for complete denaturation and solubilization of protein. After that 200 μl of 6 M NaCl was added followed by centrifugation at 11300 g for 20 min in the microcentrifuge. Subsequently, the supernatant was transferred to a 2 ml tube. Then twice the volume of 100% ethanol was added for complete precipitation of mtDNA pellet followed by washing the pellet twice with 70% ethanol. It was then dried and finally dissolved in 200 μl of sterile water. The concentration of DNA was assessed by taking nanodrop readings at 260-280nm, checking the quality of DNA by agarose gel electrophoresis.

**PCR amplification of MT-ATP6 gene:**

The MT-ATP6 gene was amplified by PCR and ATPase region was carried out in 25 μl total reaction volume, each containing 100 ng of template DNA, 0.25 pM of each primer, 2.5 μl of 10X PCR buffer, 1.5 mM MgCl2, 200 mM dNTPs, and 1.5 U of Dream Taq green DNA polymerase. PCR volume was heated initially to 95 °C for 5 mins followed by 30 cycles each consisting of 1 min denaturation at 95 °C, 40 s annealing at 56 °C, 1 min extension at 72 °C. The
reaction ended with a final extension step by incubating at 72 °C for 5 min. The PCR products were subjected to electrophoresis in a 1.2 % Agarose gel in 1X TBE buffer, stained with Ethidium Bromide (EtBr).

Sequencing Analysis:
All PCR products were sequenced using the previously reported primers. The sequence analysis were compared to the human mtDNA reference sequence using the BLAST sequence analysis tool. Mitochondrial genome sequence variations were identified using the Mitomap database. Each polymorphisms obtained were then verified against the Mitomap database. Sequence variants that were not found in that database were classified as new polymorphism/mutation, whereas others already reported were classified as reported polymorphism/mutation.

Data Analysis:
Statistical analysis was performed using SPSS 13.0 Windows Software. Mean and Standard Deviation were used for continuous variables. To quantify the correlation between survival time and each independent feature, a 95% confidence interval (CI) was used. All values were considered to be significant at p <0.05 level throughout the study.

Declarations:

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Author's Contribution:
I.M., D.V., K.J., designed the study, acquired the data and wrote the paper, I.M. and K.J. conceived of the reported analyses and performed these, G.B., S.R., helped acquire the data, M.D.S and N.S.K. oversaw the protocol and constructed the methodology, V.B., N.S.K., M.D.S., K.S.S., N.A. sanctioned the study and edited the paper. All authors viewed and approved the final manuscript and had the opportunity to comment on earlier drafts.

Competing Interests:
The authors declare that they have no competing interests.

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