

# Ovarian Carcinogenesis, underlying mutations and Diagnostic modalities

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**ABSTRACT:** The word ovarian cancer encompasses a set of tumors with different histology, clinical as well as pathological features, Precursor lesions, and underlying mutations, course of development, prognosis and response to chemotherapy. Though it does not contribute much to overall cancer burden but it is one of the deadliest cancer because of its initial silent and symptomless nature of development. There is no single mechanism elucidating its etiology being a heterogeneous group of disease. Time to time, various researches came into existence explaining mechanism of its development but a clear mechanism did not come into light. Now with increase in studies on high risk women undergoing BRCA related prophylactic Salpingo-oophorectomy, the precursor lesions and mechanism of its development is getting clearer. Here, we will explain pathogenesis, characteristics, site of origin, underlying molecular drivers of alterations of various types of ovarian neoplasms and diagnostic approaches. This knowledge will be helpful in designing effective targeted treatment and early diagnosis for ovarian cancer.

Key words: Ovarian cancer, mutations, Diagnostic modalities

**I. Introduction:** Among various Cancers, Ovarian cancer is the 8<sup>th</sup> prevalent reason of death among females and is the 7<sup>th</sup> prevailing cancer. Ovarian cancer is not an individual disease rather this term include diverse group of tumours affecting Ovaries, Fallopian tubes and Primary peritoneal cavity. Ovarian cancer is a fatal malignancy due to lack of efficient preliminary screening techniques along with appropriate biomarkers. In 2018, Ovarian cancer resulted in approximately 1,84,799 deaths constituting 4.4% of the total cancer associated fatality among females.<sup>1</sup> By the year 2035, incidence is estimated to increase to 371,000 and death rate will rise by 67% to 254,000. In India, between year 2012-2014 incidence rate (age adjustable for 100, 000) of ovarian cancer ranged from 1.7 to 15.2 in various population deployed registers of cancer.<sup>2</sup> According to human development index Asia 2012, India with 26834 cases stood second after China with 34575 annual cases. India possess world's second highest ovarian cancer occurrence as per reports of world ovarian cancer coalition Atlas 2018.<sup>3</sup> The prevalence of ovarian cancer shows different patterns globally.<sup>4</sup> The reason for this epidemiological variance in distinct areas can be accredited to the risk factors associated with its occurrence.<sup>5</sup> The highest prevalence (12.0 per 100,000), is found in non-Hispanic white women, after that Hispanic (10.3 per 100,000) followed by (9.4 per 100,000) non-Hispanic black, and (9.2 per 100,000) Asian/ Pacific Islander women.<sup>6</sup> The mortality rate owing to this cancer shows different trend may be due to dissimilarity in availability and approaches related to diagnosis and therapy. African populations showed highest mortality rate.<sup>7</sup>

**II. Staging of Ovarian cancer:** Staging is a process to figure out extent of cancer in the body. It will determine seriousness of the disease and helps to calculate survival statistics and to choose appropriate therapeutic approach. Tissue removed after surgery is examined and staging is done. A careful and accurate staging is must for better outcomes.<sup>8</sup> The most commonly followed system is FIGO (The International Federation of Gynecology and obstetrics) staging.<sup>9</sup> (Table1)

**III. Grading of Ovarian cancer:** Grading refers to change in morphology of the cancer cell microscopically. This is also given the term differentiation. In low grade cancer (well differentiated), there is lesser change in outlook of the cell. They almost appear normal but in high grade (poorly-differentiated), cells are quite abnormal. These tumors spread faster than low grade cancer. A number is assigned. A lower number like G1 is assigned for low grade and so on. Though, grading does not have effect on stage of cancer but carries importance in terms of defining treatment.<sup>8</sup>

**IV. Classification:** Ovarian cancer is classified according to tissue of origin, stage and grade of the disease. Depending upon tissue of origin WHO classified ovarian tumors majorly into epithelial surface tumors, Ovarian Germ cell tumors and Sex cord stromal tumors. Germ cell tumors originate from primordial germ cells and constitute 25% of total ovarian tumors.<sup>10</sup> Sex cord stromal tumors constitute around 7% of total tumors and arise from any of the cell types like Theca cells, granulosa cells, sertoli leydig cells, stromal cells).<sup>11</sup> Among these, epithelial surface tumors are most prevalent contributing 65% of ovarian tumors. Histologically, Epithelial tumors are further classified into types like Serous (high grade 70% and low grade 5%), mucinous 3-4%, clear cell 6-10%, endometrioid 10% and Transitional cell tumors 10% which were considered initially as subtype of Brenner tumor are now considered as morphological variant of high grade serous carcinoma and Brenner tumor as low grade carcinoma.<sup>9,11,12</sup> Serous carcinoma is most prominent. High and low grade serous carcinomas display completely different characteristics in terms of development, genomic landscape and prognosis.<sup>13</sup> (Table2)

TABLE :1 FIGO Ovarian cancer Staging

Tumor stage	Sub-stage	Explanation of Tumor
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<p>STAGE: I Tumor is restricted to ovary/ovaries or fallopian tubes (FTs).</p>	IA	Just one ovary or fallopian tube is afflicted. Tumor is absent on exterior of Ovary or Fallopian tube. Peritoneal washings and ascites are devoid of cancerous cells.
	IB	Both the Ovaries along with the fallopian tubes are afflicted. Tumor is absent on exterior of Ovary or Fallopian tube. Peritoneal washings as well as ascites are devoid of cancerous cells.
	IC	Tumor is either restricted in one or involve both ovaries or fallopian tubes with any of the following: IC 1: Capsule breakage during surgery leading to leakage i.e Intraoperative Surgical spill IC II: The ovarian capsule is ruptured prior to surgery or the tumor is found on surface of ovary and fallopian tube. IC III: Cancerous cells are detected in peritoneal washings and ascites
<p>STAGE: II Tumor incorporates one or two of the Ovaries and FTs with expansion to pelvis below pelvic brim or primary peritoneal cancer</p>	IIA	The tumor has protracted and embed into the uterus and/or FTs
	IIB	The tumor has reached to other intraperitoneal tissues in pelvis.
<p>STAGE: III Tumor incorporates one or two of the ovaries or FTs (cytological or histologically) and has confirmed dissemination to peritoneal surfaces (pelvic and abdominal) and to retroperitoneal lymph nodes</p>	IIIA	Cancer disseminates to retroperitoneal lymph nodes. (cytological or histological proven) IIIA1: a) Metastasis $\leq$ 10mm in largest dimension IIIA1: b) Metastasis $>$ 10mm in largest dimension
		IIIA2: Microscopic deposits of cancer has incorporated abdominal lining (peritoneum) outside pelvis and retroperitoneal lymph nodes might be positive.
	IIB	Macroscopic peritoneal metastasis outside the pelvis $\leq$ 2 cm in largest dimension and might be involving retroperitoneal lymph nodes. No spread to inside of liver and spleen and far off areas .
	IIIC	Macroscopic peritoneal metastasis outside pelvis $>$ 2 cm in largest dimension and might be involving retroperitoneal lymph nodes. Involves expansion to surface of liver and spleen without involving parenchyma of either organ.
<p>STAGE: IV Metastasis to distant areas outside peritoneal cavity involving parenchyma of liver/spleen and extra-abdominal organs.</p>	IVA	Malignant cells are present in Pleural effusion (positive cytology) without involving other areas.
	IVB	Cancer has involved Parenchyma (liver and splenic), extra abdominal organs and lymph nodes apart from retroperitoneal lymph nodes.

**V. High – Grade Serous Ovarian Carcinoma (HGSOC):** High grade serous carcinoma is prevalent and alone constitutes approximately 70% of ovarian cancer cases.<sup>14</sup> Here; Tumor confined to ovary is rare. They are initially unstable, aggressive and are often detected at advanced stages with bilaterally involved ovaries, peritoneal carcinosis with omental involvement making prognosis poor. It is fatal and contributes 80% of mortality from ovarian cancer.<sup>9, 15</sup>

**Molecular alterations in high grade serous carcinoma:** TCGA project has analyzed miRNA expression, mRNA expression, promoter methylation of DNA sequences of 489 high grade ovarian cancer samples, whole coding sequences of 316 of these tumors and put forth comprehensive integrated profile of varying alterations in HGSOC. According to this, TP53 gene mutations are seen in majority of 96% of cases and BRCA1 and BRCA2 germ line mutations may be found in 22% of cancers. Beside that somatic or epigenetic defect in HRR pathway meant for homologous recombination and DNA repair, somatic copy number variations and hyper methylation of the promoter genes like BRCA1 promoter is also seen.<sup>16, 17</sup> Most of TP53 mutations are missense variants. However, around 30% shows frame-shift, non-sense and splice junction mutations accounting for complete loss of p53 (p53 nulls).<sup>18</sup> p53 has a role in various processes such as repair of damaged DNA, cell cycle block, apoptosis and senescence.<sup>19</sup> Whereas, BRCA1 and BRCA2 anti-oncogenes are involved in repairing damaged DNA and are located in chromosome 17q and 13q respectively.<sup>20</sup> BRCA-linked cancers evolve 15 years prior to their non-heritable analogue as people having mutations already possess one mutated copy of the gene and require just one mutation for beginning the process of oncogenesis.<sup>21</sup> Various molecular studies postulate that high grade serous ovarian oncogenesis is triggered by initial loss of p53 and later BRCA loss results in interruption in repair of DNA and subsequently chromosomal instability. Copy number Alterations (CNA) constituting gene amplification and gene deletion is the major element behind advancement to HGSOC.<sup>22</sup>

**Precursor lesions of High grade serous carcinoma:** The ovary is lined by a monolayer of epithelium, called the ovarian surface epithelium (OSE) considered as special type of mesothelium. Earlier, view was that High grade serous ovarian carcinoma originates from OSE as result of ovulation leading to follicular rupture and release of reactive oxygen species that may predisposes to damage in DNA of cells lining OSE.<sup>23,24</sup> Fathalla in 1971 proposed a theory of “incessant ovulation” that declares that continuous ovulation lacking any intervention can lead to OC. Ovarian epithelium bears an injury or trauma during ovulation followed by repair. Wound is repaired by multiplication of epithelial cells and their assemblage form cortical inclusion cysts. There is plausibility of DNA damage leading to malignant transformations in the whole process resulting in Mullerian metaplasia of the coelomic epithelium.<sup>25</sup> Though precursors were thought to present within ovary itself but none of the studies in the past three decades found convincing precursor lesions inside. The precursor lesions were found within the fallopian tube during prophylactic salpingo-oophorectomy of high risk women with BRCA1 and BRCA2 mutations. The fallopian tubes were removed, sectioned and examined using an advanced technique called “sectioning and extensively examining the fimbriae technique”.<sup>26</sup> These precursor lesions are studied as STIC (serous tubal intraepithelial carcinoma) and begin in distal end of fimbriae. A great percentage of HGSOC (~80%) has tubal origination, on other hand, primary peritoneal HGSCs are extremely rare.<sup>27</sup> While addressing serous tumors, its tubal carcinogenesis is admitted but some percentage of high grade serous carcinomas lack tubal precursor lesion. So, for certain percentage of serous cancers, the older concept of pathogenesis may still apply. A well founded point of origin in advanced serous carcinomas is not achievable as tumour has metastasized to nearby tissues bewildering the main site.<sup>28</sup>

**Pathogenesis of High Grade serous carcinoma:** The early event underlying pathogenesis of High grade serous carcinoma is TP53 mutations especially in benign-looking secretory cells. These precancerous lesions are termed as ‘p53 signatures’. Obtaining a neoplastic appearance and an ability to multiply lead to evolution of serous tubal intraepithelial carcinoma (STIC). Encroachment of the basement membrane and the limited spread involving ovary and/or peritoneal cavity leads to invasive HGSOC. HGSOCs involving the ovary or peritoneum carry alterations in TP53 gene (and BRCA1 in familial cases) and show copy number variations.<sup>29</sup> STIC found in distal fimbrial epithelium of fallopian tubes of women with sporadic HGSOC (21-59%) and (3-31%) with genetic HGSOC. The origin of HGSOC from STIC is supported by the fact that the molecular profiles of HGSOC are identical to fallopian tube epithelium (FTE) compared to OSE.<sup>30</sup> Moreover, upregulated expression of p53 is same as that of HGSOC. STIC also manifest Ki67 proliferation index greater than 10%.<sup>31</sup> STIC may be underlying cause of ovarian or pelvic HGSC but not of other carcinomas like endometrioid, mucinous and clear-cell. Some researchers have put forward that HGSCs may originate in two separate pathways.<sup>32,33</sup> One with the classic HGSC arising from a STIC and the SET variant originating alternatively from STIC, some other tubal precursor, or elsewhere.<sup>34</sup> The usual or classic type is characterized by compact masses of cells having slit-like areas, papillary, glandular, and cribriform configuration frequently associated with necrosis. Cells display noticeable anisonucleosis with conspicuous nucleoli, raised mitotic potential as well as atypical mitosis. SET type constitutes solid masses of cells that provoke endometrioid carcinoma along with transitional cell carcinomas. Micropapillae and bizarre giant cells may also be spotted. It shows larger amount of tumour infiltrating lymphocytes and great percentage of cells in mitotic phase i.e mitotic index.<sup>35,36</sup> This type have similar immunoprofile as usual HGSC like p53, PTEN, WT1 responsiveness and an identical rate of TP53 alterations<sup>15</sup> but SET tumors were associated mostly with BRCA1/2 mutations.<sup>36</sup>

**VI. Low Grade serous ovarian carcinoma (LGSOC):** In terms of both morphological and molecular aspects, LGSOC is completely dissimilar from HGSOC constituting around 3% of ovarian cancers. They are genetically stable tumors with low malignant potential and are slower in development but advanced stage diagnosis have worst prognosis being poorly perceptible to established platinum based chemotherapy.<sup>37</sup>

**Pathogenesis of LGSOC:** Studies are confirming that LGSOC are incepted from fallopian tube epithelium, earlier considered to arise from ectopic mullerian epithelium on the uppermost layer of ovary.<sup>38</sup> They develop from benign and borderline serous tumors and before malignant transformations, undergo a sequential progression to adenoma and then atypical proliferative tumor.<sup>39,40</sup> Mutations results in progression of benign form through borderline to non-invasive form and then further into invasive low grade carcinoma. There is no proof of evolution to high grade serous carcinoma.<sup>41,42</sup> In LGSC, cancer cells present abnormal looks of nuclei i.e nuclear atypia without anisonucleosis and noticeable nucleoli. Mitoses is not greater than 12 per 10 hpf (high power fields) with recurrent psammoma bodies. Ki 67 proliferation index is small (<3%) and relates with poor prognosis. In contrast to Serous borderline tumor, LGSC displays stromal invasion more than microinvasion.<sup>43</sup>

**Molecular variations in Low grade carcinoma:** Mutations in KRAS and BRAF are most common among low grade serous carcinoma constituting around two-third of LGSOC cases. Mutation in KRAS present with more hostile and reiterative disease compared to mutation in BRAF.<sup>44</sup>This may be linked to mutations in mitogen activating protein kinase (MAPK) pathway involved in transmission of growth signals in the nucleus.<sup>12</sup> LGSOC exhibits MAPK pathway-activating mutations in KRAS or BRAF gene (approximate contribution 30% each), but do not show TP53 mutations.<sup>44</sup>Unlike HGSC, LGSC do not show BRCA germ line mutations and chromosomal instability.<sup>27</sup>

Table:2 Five Major Types of Epithelial Ovarian cancer

	High Grade Serous	Endometrioid	Clear-cell	Low Grade Serous	Mucinous
Origin	Fallopian tube epithelium	Endometriosis	Endometriosis	Fallopian tube epithelium	Not Known
Precursor lesion	STIC	EBOT	CCBOT	SBOT	MBOT
% contribution to Ovarian cancers	70% approx..	10% approx.	6-10% approx.	<5%	3-4% approx..
Underlying Mutation /Alteration	TP53,BRCA1/2, defect in HRR pathway	PTEN, CTNNB1, ARIDIA and PP2RIA	PIK3CA, CTNNB1, ARIDIA and PP2RIA	KRAS,BRAF	KRAS, amplification of HER2

**EBOT: Endometrioid borderline tumors, CCBOT: Clear-cell borderline tumors, SBOT: Serous borderline tumors, MBOT: Mucinous borderline tumors**

**VII.Endometrioid Carcinoma (EC):**Endometrioid tumors constitute approximately 10-15% of all ovarian tumors.These tumors evolve from borderline/atypical proliferative tumors and are associated with endometriosis and regions of endometrioid adenofibroma and endometrioid borderline tumor. In about 40% of Endometrioid Carcinomas, atypical endometriosis entitled to be precursor lesion. The endometriotic tissue associated with neoplasia may originate de novo due to molecular alterations or might have transported from endometrium. Most endometrioid carcinomas are low grade and are discerned in initial stage.<sup>45,46</sup> High grade endometrioid carcinoma is now categorised as SET variant of HGSC as it imitate high grade serous carcinoma. High grade endometrioid are poorly differentiated tumors that display solid growth and distinct atypical cells and high mitotic activity.<sup>47,48</sup> Low grade endometrioid carcinoma may not be histologically same but their pathogenesis and molecular features are same as that of clear cell carcinoma.<sup>27</sup>

**Molecular alterations in Endometrioid tumor:** The genomic landscape of ovarian EC is indistinguishable from its endometrial counterparts.<sup>44</sup> Endometrioid tumors have somatic mutations in PTEN, POLE exonuclease domain and ARIDIA. But, CTNNB1 is most common mutation in endometrioid carcinoma. There occur alterations in wnt/ $\beta$ -catenin signalling pathway due to mutation in catenin  $\beta$  1 (CTNNB1) gene that encodes PTEN and  $\beta$ -catenin. PTEN mutations occur at a low rate constituting around 20% of cases.<sup>49</sup>CTNNB1 mutation is seen in 38-50% of cases culminating in aggregation of  $\beta$ -catenin in both nucleus as well as cytoplasm.  $\beta$ -catenin has a role in cell adhesion, movement and viability.<sup>50</sup>  $\beta$ -catenin mutation leads to squamous differentiation, a feature of Endometrioid carcinoma.<sup>51</sup> Instability in microsatellites is also present in EC.<sup>52</sup> PTEN is an antioncogene. Somatic alterations in PTEN and heterozygosity loss sometimes coincides triggering PI3K/AKT pathway that prevents programmed cell death.<sup>49</sup> ARIDIA mutations are present in around 30% of ovarian EC. ARIDIA (AT rich interactive domain 1A) also behave as antioncogene and codes for protein BAF 250 a , a part of SW1/SNF which is a multiprotein chromatin remodeling complex that upregulates or suppress transcription.<sup>53</sup> PP2RIA gene mutations are also seen in minority that encodes subdivision of protein phosphatase 2A.<sup>54</sup> A subtype of EC analogous to HGSC bears TP53 mutation, Homologous recombination deficiency mutations and copy number alterations. This subtype often identified at advanced FIGO stages( III and IV) and have poor prognosis.<sup>52,55</sup>

**VIII.Clear-cell Carcinoma (CCC):** Clear cell carcinoma constitutes 6-10% of total ovarian cancers. Same as that of endometrioid carcinoma, endometriosis is the major reason behind clear cell cancer development but some regions of adenofibroma and borderline tumors are also found. Studies shows that tumors with component of adenofibroma have better prognosis than non-adenofibromatous component.<sup>56</sup>Unlike endometrioid, clear cell carcinoma presents as unilateral masses. It is often identified at onset compared to high grade serous carcinoma and around 25% of tumors detected at early FIGO stage (I and II) are constituted by CCC.<sup>14</sup>But advanced stage of the disease has poor progression free as well as overall survival as it shows intrinsic resistance to platinum therapy.<sup>57</sup>Cancer cells exhibit clear eosinophilic cytoplasm and non-typical nuclei with distinct nucleoli but unaccompanied by notable pleomorphism. Mitoses is generally below 5/10 high power fields.<sup>46, 48</sup>

**Molecular alterations in Clear-cell Carcinoma:** Loss of function mutation resulting decreased protein function of ARIDIA1 gene is most common alteration found in nearly half of all ovarian clear cell carcinomas and are associated with atypical endometriosis.PIK3CA (phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit  $\alpha$ ) stimulating mutations constitute around 30-40% of all ovarian CCC along with ARIDIA1.<sup>58</sup> PIK3CA codes p110a catalytic subunit of PI3K leading to stimulation of PI3K/AKT pathway meant to inhibit programmed cell death and encouraging proliferation of cells. It also resists effect of PTEN, a tumor suppressor opponent of the pathway.<sup>59, 60</sup> Around 5-20% of CC OC has mutations in PTEN and loss of heterozygosity among other alterations.<sup>61</sup> Mutation in BRCA1, BRCA2, TP53 are less likely. Alterations in CTNNB1 are oblivious unlike to endometrioid carcinoma. Germline mutations in MMR (mismatch repair) genes: MLH1 (Mut L Homolog 1), MSH2 (Mut S Homolog 2), MSH6 (Mut S Homolog 6), or PMS2 (PMS1 Homolog 2) results in deficient expression of mismatch repair associated with Lynch syndrome.

<sup>62,63</sup>This autosomal dominant disorder results in instability in microsatellites inclining to risk of early initiation of ovarian cancer. A study showed that 20% of cases with EC and CC OC has MMR deficient tumors.<sup>64,65</sup>

**IX. Mucinous Carcinoma (MC):** Mucinous carcinoma constitutes around 3% of all epithelial tumors. The pathogenesis of mucinous carcinoma is still not acknowledged much. A number of speculations have been put forth. Kurman and Shih proposed that site of their emergence is epithelium of tuboperitoneal junction and predecessor lesions are Brenner tumors which are mostly benign rarely malignant.<sup>19</sup> Benign tumors (mucinous cystadenoma) may sequentially advance to mucinous borderline tumour or atypical proliferative tumor and then to mucinous carcinoma.<sup>66</sup> Most mucinous tumors unveils gastrointestinal differentiation and seldom display endocervical differentiation. Tumors in first stage have good prognosis. Spread to extraovarian regions worsen prognosis.<sup>26</sup>

**Molecular alteration in mucinous carcinoma:** Commonest alteration is copy number loss of CDKN2A (76%) ensued by KRAS and TP53 mutation (64% combined). CDKN2A codes p16 and p14 that act as suppressors of tumour. Subsequently, recurrent alterations like HER2 amplification, mutation in ARID1A, RNF 43, BRAF, PIK3CA are also seen.<sup>67</sup> Copy number alteration is a core factor linked with rising grade and advancing metastasis.<sup>26</sup>

**X. Diagnostic modalities for Ovarian Cancer detection:** Late stage diagnosis of ovarian cancer is a common problem and when the symptoms appear, the disease has already progressed; therefore, early stage detection becomes very difficult as it is often asymptomatic. Absence of effective specific markers and/or lack of effective tools cause delayed early stage diagnosis and subsequently the prognosis of ovarian cancer patients.

**Ultrasound for Ovarian Cancer:** Detailed image of ovaries and their morphological variations can be obtained with ultrasound by which the developing malicious cells can be identified. The transvaginal route is mostly preferred. The data including size of ovaries, abnormal lesions, pelvic or abdominal fluid, and flow of blood in the ovaries can be obtained. The power color dopplers are used to know the diagnostic variables such as volume of the ovary, the structure of the cyst wall, papillary vegetation, septation and echogenicity as transvaginal ultrasound (TVUS) findings to predict cell abnormality and to detect ovarian cancer in the early stages. An important limitation in ultrasound diagnosis includes observers having varying interpretations and thus, ultrasonography images are scored differently.

**MRI in Ovarian Cancer Detection:** Magnetic resonance imaging (MRI) is commonly employed technique for assessing gynaecological disorders. Images with higher resolution can be captured rapidly. To improve the image acquisition process, Phased array coils compatible with parallel imaging approaches are used. The imaging speed is increased as these approaches use spatial information from the elements of a radiofrequency (RF) receiver coil array. The MRI images taken for detecting ovarian cancer must contain the entire abdomen and pelvis. Generally, images are captured in an axial plane. To determine the spread, the liver surface, diaphragm and pelvic sidewall have to be examined with the coronal images. In the pelvis, sagittal sequences present the relation among ovarian neoplasm, uterus, bladder and rectum.

**Biomarkers Or Tumor markers:** These are basically products of cancer cells removed in circulation and can be detected. They can be used for differential diagnosis, prognosis for observing effect of treatment and for determining recurrences.<sup>68</sup> Ideally these biomarkers are collected non-invasively from biofluids like blood and serum and assayed for presence of cancer in the human body. In patient care already several protein biomarkers are in use. More than half of malignancies are curable if diagnosed early.

**Cancer Antigen 125 (CA-125):** Most of the epithelial ovarian tumours express biomarker cancer antigen 125. It is a glycoprotein acquired from epithelium of coelomic and mullerian origin, belonging to mucin family encoded by MUC 16 gene.<sup>69</sup> In 93% cases with ovarian cancer, CA-125 levels corresponds with tumor load. In advanced ovarian cancers, elevated levels are seen in 80-90% of women but in early stage i.e I stage of the disease only 50% of women shows raised levels.<sup>70</sup> Researchers have shown that serial measurements of CA-125 can prove effective. Care must be taken to note down the false-positive results in medical disorders.

**Human Epididymis Protein-4(HE4):** It is an upcoming biomarker for differential diagnosis of epithelial ovarian cancer from benign ovarian masses. It is a glycoprotein of 20-25 KDA found in epithelial lining of distal epididymis during initial detection. The first study by Hellstrom et al concluded that HE4 has better specificity than CA-125 and equivalent sensitivity to CA-125 in detecting ovarian cancer.<sup>71</sup> Moreover Midulla et al found that raised up serum HE4 levels prior to treatment substantially associated with high tumor grade, serous histology and peritoneal relationship, nodal intrusion, stage and size of tumor etc.<sup>72</sup>

**Liquid Biopsy:** Liquid biopsy is in great demand in clinical practice now a days. The use of Circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), circulating cell-free microRNAs (cfmiRNAs) and circulating exosomes represent the major components of liquid biopsy. The advantage of using these cell-free circulating components as a source of liquid biopsy lies in the fact that they enter into circulation from the point tumor becomes cancerous. Although liquid biopsy is not implemented for ovarian cancer diagnosis but most of the researches already done were focussed on ctDNA and cfmiRNAs. miRNAs as potential candidates for liquid biopsy are being explored these days.<sup>73</sup> The, dysregulated expressions could serve as minimally-invasive procedure to screen ovarian cancer patients, as this dysregulation could be explored much earlier than the appearance of clinical symptoms. Most importantly, due to its minimally-invasive approach, injury to the patients is avoided, as in the case of tissue biopsy or other examination/diagnostic procedures. Therefore, differential expression of circulating miRNAs could serve as minimally-invasive procedure for early ovarian cancer detection. miRNAs are endogenous small non-coding RNAs 19-25 nucleotides in length. About 60% of human genes are under regulation of miRNAs. They either directly target 3' untranslated region (3'UTR) of cognate messenger RNAs (mRNA) of protein coding genes and results in its degradation or translational repression. In non-protein coding genes, miRNAs bind either to the microRNA response elements or the 3'UTR of non-coding RNAs.<sup>74</sup> Many researches have reported that miRNAs act as key regulators and regulates the post-translational cell machinery contributing in cancer development via cell proliferation, cell differentiation, cell fate, apoptosis, invasion, angiogenesis, signal transduction, and epithelial-to-mesenchymal transition (EMT). Their expression profiles have shown tissue specific patterns and are found to be dysregulated in various cancers in

comparison to normal individuals. They can act as either tumor suppressors or oncomiRs or both, depending on the cancer-type. Thus, their differential profiles could be explored through quantitative real-time polymerase chain reaction (qRT-PCR), Northern blotting, microarrays or through deep sequencing for their importance in clinical manifestations .

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