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REVIEW

Safety of clomiphene citrate: a literature review

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Abstract Clomiphene citrate (CC) is a nonsteroidal compound and induces ovulation indirectly. The wide usage of the CC raises a question; is it safe or not? In the light of this question, this review aimed to highlight all researches and insights into the association between the use of CC and risk of genotoxicity, cytotoxicity, embryotoxicity, teratogenicity and risk of different cancer types. We conducted a MEDLINE/PubMed, Scopus, Web of Science, Google Scholar search. After a careful screening process of all authors, 32 of these articles were considered as appropriate, and reviewed. Our evaluations showed that CC has genotoxic, cytotoxic, embryotoxic and teratogenic properties. There is no association between the use of CC and risk of ovarian, breast, uterine, cervix, endometrium, lung, colorectal cancer, and lymphoma. However, risk increased especially after 6 cycles of use and especially in nulligravid women. The use of CC should be restricted to 6 cycles. Moreover, malignant melanoma and thyroid cancer risk was found to be higher among CC treated women in almost all studies. Further works should be conducted especially in animal models to assess its risk features.

Keywords Clomiphene citrate · Genotoxicity · Cytotoxicity · Embryotoxicity · Teratogenicity · Carcinogenicity

Introduction

Clomiphene citrate (CC) is a nonsteroidal compound and induces ovulation indirectly. It has been used for decades in ovulation induction and assisted reproduction. Especially effective for the treatment of anovulatory patients and polycystic ovarian syndrome (PCOS) (Fiedler and Ludwig 2003; Trabert et al. 2013). PCOS is a disorder characterized by oligoovulation and hyperandrogenism. PCOS confers risk of infertility, obesity, insulin resistance and diabetes (Prince et al. 2016).

CC is also used for male infertility due to spinal cord injury and multiple sclerosis and gynacomastia in both adolescent and pubertal males (Plourde et al. 1983; Brackett et al. 1995; Ara and Asmatullah 2011). Furthermore, CC was found to be effective for long-term therapy for male hypogonadism by increasing testosterone level (Moskovic et al. 2012). CC antagonizes the negative feedback of estrogen at the hypothalamus with a consequent increase in ovarian stimulation by endogenous gonadotropin and

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subsequent rises in circulating follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels (Legro et al. 2014; Athar et al. 2015). CC is administered orally, typically starting on the third to fifth day after the onset of spontaneous or progestin-induced menses. Treatment begins with a single 50-mg tablet daily for 5 consecutive days. The effective dose of CC ranges from 50 to 250 mg/day; doses in excess of 100 mg/day are not approved by the FDA (ASRM 2006; Yilmaz et al. 2014). CC is absorbed in gastrointestinal tract, metabolized in liver and generally well tolerated in human body. Drug and its metabolites are not changed by liver and are excreted in the feces. The biological half-life is 5–6 days; however, its metabolites have been found in feces up to 6 weeks (Ara and Asmatullah 2011). Some transitory side effects like hot flashes, mood swings, headaches and visual disturbances were reported (Hughes et al. 2000).

CC is widely used in a world-wide basis since 1967 and the main advantages of this drug are its low price and the almost negligible risk of ovarian hyperstimulation (Fiedler and Ludwig 2003). The wide usage of the CC raises a question; is it safe or not? In the light of this question we decided to evaluate its safety features especially in view of its cytotoxicity, embryotoxicity, teratogenicity, genotoxicity and carcinogenicity.

Methods

Data sources

Owing to the medical nature of the question, in this review, we conducted a MEDLINE/PubMed, Scopus, Web of Science, Google Scholar search of the terms alone or in combination of "fertilization in vitro", "intracytoplasmic sperm injection", "ovulation induction", ''clomiphene citrate'', "breast", "ovarian", "uterine", "melanoma", "thyroid", "nulligravid", ''toxicity'', ''genotoxicity'', ''carcinogenicity'', "cytotoxicity", "embryotoxicity", "teratogenicity", ''cancer'' and ''safety''. This systematic review included all toxicity, genotoxicity and carcinogenicity data investigated with the Clomiphene Citrate.

Study eligibility criteria (limitations)

The results were restricted to articles written in English. We excluded case reports, biochemistry results in cell cultures, in animal models and in humans. Over 100 abstracts published up to June 2016 including studies in drug information, case reports, retrospective cohort studies, reviews, animal studies and in vitro cell studies were found. Whenever multiple publications of the same data set are realized, only the most recent and/or complete study was included. After a careful screening process of all authors, 32 of these articles were considered as appropriate, and reviewed.

Results

Genotoxicity, cytotoxicity, teratogenicity and embryotoxicity

The genotoxic effects of Clomiphene citrate are poorly investigated (Table 1). Few in vitro and in vivo works were published and argued its genotoxicity potential. Ohnishi et al. (1986) investigated Clomid, containing Clomiphene citrate, mutagenicity in *Escherichia coli* radiation-sensitive mutants, uvrA and recA, and wild-type strains. The authors reported that radiation-sensitive mutants were more sensitive to CC than wild type strains and it caused DNA strand breaks. In another work, CC was found to be inducer of frameshift mutations in the *Salmonella typhimurium* TA1538, TA97 and TA100 strains but not of base pair substitution mutations. These results were partly supported by Yilmaz et al. (2014). On the contrary, Ohnishi et al. (1986) found that CC induced genolethal DNA damages in the *Escherichia coli* PolA−/PolA+ with S9. Duran et al. (2006) reported that CC significantly and dose-dependently increased micronucleus frequency in bone marrow cells of rats. In a work of Yilmaz et al. (2014), CC genotoxicity was measured by bacterial and mammalian test systems. CC showed no genotoxicity in *E. coli* TA97 and TA100 strains, however it significantly induced chromosomal aberrations, DNA damage and micronucleus in human peripheral blood lymphocytes. Authors further added an investigation published in 2015 regarding CC induced Sister Chromatid Exchange (SCE). Increased SCE frequency was observed for

Table 1 Genotoxic, cytotoxic, teratogenic and embryotoxic effects of clomiphene citrate

Test Substance	Test material	Results	References
Clomid (containing clomiphene citrate)	*E. coli* uvrA, recA and wild-type	Increased DNA strand breaks	Ohnishi et al. (1986)
Clomiphene citrate	*S. typhimurium* TA 97, 100, 1538	Incraesed frameshift mutations no icrease in base pair substitution type mutations	Ohnishi et al. (1986)
Clomiphene citrate	*E. coli* PolA− PolA+	Increased genolethal DNA damage	Ohnishi et al. (1986)
Clomiphene citrate	Rat bone marrow cells	Increased micronucleus formation	Duran et al. (2006)
Clomiphene citrate	*E. coli* TA 97, TA 100	No evidence for Frameshift mutations and base pair substitution type mutations	Yilmaz et al. (2014)
Clomiphene citrate	Human lymphocytes	Significantly incraesed chromosomal aberrations micronucleus sister chromatid exchanges	Yilmaz et al. (2014)
Clomiphene citrate	Human prostate cancer cells	Mild cytotoxicity	Jiann et al. (2002)
Clomiphene citrate	Breast cancer cells	Apoptotic	Elloumi-Mseddi et al. (2015)
Clomiphene citrate	Human peripheral blood mononuclear cells	Cytotoxic	Costa et al. (2012)
Clomiphene citrate	Pregnant mouse	Embryotoxic teratogenic	Ara and Asmatullah (2011)

tested concentrations (Yilmaz et al. 2015). Cytotoxicity mechanism of CC was also investigated in human prostate cancer cells. CC was found to increase cytosolic Ca^{++} level and cause mild cytotoxicity (Jiann et al. 2002). Furthermore, in a recent research by Elloumi-Mseddi et al. (2015) CC was found to be a proapoptotic drug and it causes cell death in breast cancer cells by phosphorylation of Extracellular Signal-Regulated Protein Kinase (Erk 1/2). The cytotoxic and antioxidative potential of CC was also reported by Costa et al. (2012). Reactive Oxygen Species (ROS) production and cytotoxicity assays were performed on blood and peripheral blood mononuclear cells from carriers of different Val16Ala SOD2 genotypes. The authors reported that CC exhibited antioxidant capacity and decreased ROS production. The ascorbic acid dominant genotype (AA) was found to be more responsive to antioxidant effect. But authors have also reported CC increased cell viability and had no cytotoxic activity.

Teratogenicity and embryotoxicity of CC in in vivo systems is also poorly documented (Table 1). There is only one article published by Ara and Asmatullah (2011). The authors administered a single dose of CC (1, 2, 4 and 8 μg/g BW) to pregnant mice on day 8 of gestation to examine morphologic, morphometric and histological changes in fetuses. Morphological changes were recorded as open eyelids, anophthalmia, fore and hindlimb micromelia, meromelia, amelia, sacral hygroma, hydrocephaly, hemorrhagic spots, kyphosis and clubbed feet. Morphometric analysis showed significant reduction in fetal body weight crown rump length, head circumference, eye circumference, forelimb and hindlimb lengths and tail size. And histological observations showed that CC caused brain defects like hydrocephaly, enlarged ventricles and undifferentiated neuroglial cells in cerebellum.

Carcinogenicity

The carcinogenicity potential of the CC is summarized in Table 2. The association between ovulation induction (especially CC) and risk of endometrial cancer was investigated in small population of 128 women living in Israel with histologically diagnosed endometrial carcinoma. The 255 controls were from the same area and the authors found no evidence about relation between the use of CC and risk of endometrial cancer (Benshushan et al. 2001). Rossing et al. (2004) investigated a larger case–control study among 2015

Table 2 Carcinogenic potential of clomiphene citrate

Study design	Cancer type	Result	References
383 women	Endometrium	No risk	Benshushan et al. (2001)
2015 women	Ovarium	No risk	Rossing et al. (2004)
12,193 women	Breast	Increased risk	Brinton et al. (2004)
8422 women	Melanoma, thyroid, colon, cervix	Increased melanoma and thyroid cancer risk among nulligravid women	Althuis et al. (2005)
54,362 women	Thyroid	Increased risk	Hannibal et al. (2008)
15,030 women	Uterus, breast, melanoma, non-hodgking lymphoma	Increased risk	Calderon-Margalit et al. (2009)
15,030 women	Ovarium	No risk	Calderon-Margalit et al. (2009)
54,362 women	Uterus	Increased risk among $\geq$ 6 cycles clomiphene citrate user	Jensen et al. (2009)
7355 women	Breast, corpus uteri, ovarium	No risk	Santos Silva et al. (2009)
18 patients	Breast	No risk	Sönmezer et al. (2010)
3091 women	Breast	Reduced risk	Fei et al. (2012)
12,193 women	Ovarium	No risk	Trabert et al. (2013)
12,193 women	Breast	Increased risk among multiple clomiphene citrate user	Brinton et al. (2014)
1073 women	Ovarian	No risk	Perri et al. (2015)
12,193 women	Lung, colorectal	No risk	Brinton et al. (2015)
12,193 women	Melanoma, thyroid	Increased risk	Brinton et al. (2015)

women aged 35–54 years (378 cases and 1637 controls) to evaluate relation between ovarian cancer and infertility/use of ovulation induction drugs. The authors have not observed any relation between the ovarian cancer and use of CC among parous women. Nevertheless, risk was shown to be increased among nulliparous women. In a larger retrospective cohort study conducted by Brinton et al. (2004) with 12.193 women evaluated for infertility between 1965 and 1988 in the USA, 292 in situ and invasive breast cancers were identified among the population. The authors reported that a statistically significant increase was observed between CC use and invasive breast cancer. They concluded that infertile patients had a significantly higher breast cancer risk than the general population.

In a large retrospective cohort study of Althuis et al. (2005), melanoma, thyroid, colon, and cervical cancer risks after clomiphene use were analyzed. Totally 8422 women were studied and 102 cancer cases were reported among either CC user or not user. The authors have reported that CC use did not significantly increase the risk of melanoma, thyroid, colon, and cervical cancer. However, increased melanoma and thyroid cancer risks were observed among nulligravid women who used CC. The results of Althuis et al. (2005) were not supported by the Calderon-Margalit et al. (2009). The authors conducted a large cohort

study to examine CC use and risk of colon, breast, ovarian, and uterine cancer, malignant melanoma and Non-Hodgkin lymphoma among 15,030 women in the Jerusalem Perinatal Study. Women who used drugs, especially CC to induce ovulation (n = 567) had increased risks of uterine and breast cancer, malignant melanoma and Non-Hodgkin lymphoma. Unlikely, no relation was observed between use of ovulation-inducing agents and ovarian cancer. In a large cohort study among Danish population (54.362 women, 83 uterine cancer case) conducted by Jensen et al. (2009). The authors showed that ever use of any fertility drug (including CC) was not associated with uterine cancer, but the risk increased with number of cycles of use of CC ($\geq$ 6 cycles). Another study was designed by Santos Silva et al. (2009) with a large British population of 7355 women who had ovulatory disorders. Nearly half of the population were ovulation induction drug users and the authors reported that no significant differences in the risk of cancers of the breast, corpus uteri, ovary, or of any other site existed, between women who had been prescribed ovarian-stimulation drugs and those who had no prescription of these drugs. Further interesting data was published in Reproductive Medicine Online. The article by Sönmezer et al. (2010) compared the tumor characteristics of 18 patients (study group) diagnosed with breast cancer within 24 months of undergoing ovarian stimulation with either gonadotrophins or clomiphene citrate. The authors found that tumor characteristics were similar in the study group and in the control group and they have concluded that there was no correlation with the use of CC and breast cancer risk. Interestingly a reduced young set breast cancer risk was reported by Fei et al. (2012). They have conducted two sister studies, a sister matched case–control study by enrolling 1422 women who were younger than age of 50 years at diagnosis with breast cancer and 1669 breast cancer free control. Totally, 288 participants who used ovulation-stimulating drugs (including CC) showed a non-statistically significantly decreased risk of breast cancer.

A retrospective cohort study in United States was designed to evaluate relationship between CC use and ovarian cancer risk by Trabert et al. (2013). A total of 12.193 women with primary or secondary infertility who sought advice for infertility between 1965 and 1988 were included in this work. The results showed that there was no association between CC use and ovarian cancer risk. The association between long term use of ovulation stimulating drugs and breast cancer risk were examined by Brinton et al. (2014). An extended follow-up cohort study among 12.193 infertile women was evaluated. 749 breast cancers were observed and no correlation was found between CC use and breast cancer. However, higher breast cancer risks were seen for patients who received multiple CC treatments.

The risk of invasive epithelial ovarian cancer among *BRCA1* and *BRCA2* mutation carriers of Jewish Israeli women who used CC was examined and no association between the use of CC and invasive epithelial ovarian cancer was reported (Perri et al. 2015). Another large cohort study was conducted by Brinton et al. to examine association of CC use and colorectal, lung, thyroid and melanoma cancers. In total 12.193 patients were screened and 35% of them were past user of CC. The authors did not find any relation in the increase of lung and colorectal cancers, but a significant increase in melanoma and a non-significant increase in thyroid cancer was found among the population. The authors have also reported that CC-associated risks for thyroid cancer were somewhat higher among nulligravid women (Brinton et al. 2015). The association of CC use and thyroid cancer was also supported by Hannibal et al. (2008). The authors designed a large cohort study among 54.362 women with infertility problems between the years of 1963–1998 in Denmark. A total of 29 women diagnosed with thyroid cancer during the follow-up period were included in the study. They observed that the use of CC was associated with an increased thyroid cancer risk (Hannibal et al. 2008).

Conclusion

Majority of the current results have shown that CC is genotoxic, cytotoxic, embryotoxic and teratogenic agent. General toxicity mechanism of CC is not known, however increase in cytosolic Ca^{++} level and Erk phosphorylation can be responsible for it's genotoxicity and cytotoxicity (Jiann et al. 2002; Elloumi-Mseddi et al. 2015; Yom-Tov et al. 2012).

When we analyzed the carcinogenicity potential of this drug there were no strong association between the use of CC and risk of ovarian, breast, lung, colorectal, cervix, endometrial cancers and Non-Hodgkin

lymphoma. Some reports have concluded relationship but majority of the results showed no risk. However, the risk increased especially after 6 cycles of use and especially in nulligravid women. That is why the use of CC should be restricted to maximally 6 cycles. Moreover, malignant melanoma and thyroid cancer risk was found to be higher among CC treated women in almost all studies. Further animal studies as well as clinical cohort investigations are certainly needed to reach a decision about its safety.

Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest relevant to this article.

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