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Review

Mechanisms of apoptosis in ovarian cancer: The small molecule targeting

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Ovarian cancer is the deadliest cancer among women. About 90% of ovarian cancers are epithelial, (ovarian carcinomas) thought to arise from the ovarian surface epithelium. Diagnosed usually at clinically advanced stages, many patients show poor response to chemotherapy, with resistance and recurrent disease being prevalent. The cell origin and the mechanism of neoplastic transformation of this malignancy are poorly understood. Apoptosis is crucial in normal ovarian development and function; and gonadotropins play a significant role in modulating the expression of several pro-apoptotic and pro-survival genes and other molecules in the ovary. Targeted therapeutic strategies using small molecule protein kinase inhibitors and monoclonal antibodies have been explored in the management of ovarian carcinomas. These molecules, used in combination with chemotherapy, have shown better prognosis in ovarian cancer. With several ongoing clinical trials using kinase inhibitors and the ideal targets being sought after, significant improvements of patients suffering with ovarian carcinomas are expected in the near future. This manuscript aims to review ovarian apoptotic mechanisms and the therapeutic progress in the use of small molecule kinase inhibitors and monoclonal antibodies as targets for inducing apoptosis in ovarian cancer.

Key words: Ovary, apoptosis, gonadotropins, targeted therapy.

INTRODUCTION

Ovarian cancer is the leading cause of death among all gynecological cancers in western countries. When compared to other gynecological malignancies, the mortality rate of ovarian cancer exceeds that of cervical and uterine cancers put together (Siegel and Jemal, 2013). This high death rate is due to the diagnosis at an advanced stage in most patients which is caused by the relative lack of specific signs and symptoms of the disease, and the lack of reliable tests for early detection. Disease-associated mortality in ovarian cancer is as a result of metastasis, which is characterized by ascites,

peritoneal and systemic dissemination.

Despite platinum and paclitaxel-based chemotherapy plus cyto-reductive surgery in ovarian cancer, less than one-third of patients will survive after 5 years (Cannistra, 2004; Zhang et al., 2009; Gavalas et al., 2010; Kim et al., 2012). There are more than 30 types and subtypes of ovarian cancer; however, they have been classified into 3 major categories according to the primary cells from which they arise. They are categorized as either: epithelial tumours, stromal tumours or germ cell tumours (Kaku et al., 2003). Epithelial ovarian tumours (ovarian

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carcinomas) are the most prevalent and the most aggressive; accounting for about 90% of all ovarian cancers (Sankaranarayanan and Ferlay, 2006; Gavalas et al., 2011).

The exact cause of ovarian cancer is not known, however, there are several factors which can increase a woman's risk of developing ovarian carcinomas. Most cases of ovarian carcinomas occurs in post-menopausal women, with the median age of women affected being over 60 years (Cannistra, 2004). Hormonal factors including multiparity, lactation and use of oral contraceptives have been associated with decreased risk. Nulliparity, the use of hormone replacement therapy and having late menopause are associated with increased risk (Schildkraut and Thompson, 1988; Gwin et al., 1990).

Studies have indicated the most predominant risk factor for ovarian cancer is having a strong family history of breast cancer, ovarian cancer or both (Cannistra, 2004). 11 to 40% of ovarian cancers are associated with germline mutations in BRCA1 and/or BRCA2 genes (Petrucci et al., 1993). While milder, less aggressive and genetically more stable ovarian cancers involve mutations of V-Ki-ras2 Kirsten rat sarcoma viral oncogene homologue (KRAS), v-Raf murine sarcoma viral oncogene homolog B1 (BRAF), Phosphatase and tensin homolog (PTEN) and B-catenin, mostly termed borderline tumours, most high grade epithelial ovarian cancers (carcinomas) such as undifferentiated tumours, high grade serous carcinomas and mixed malignant mesodermal tumours are as a result of TP53 mutations (Kurman and Shih, 2008). About 50% of human cancers have shown inactivation of p53 through mutations or loss. In most characterized tumours retaining wild type p53, disruptions have been found in the p53 pathway of tumour suppression (Harris and Hollstein, 1993). P53 gene mutations are frequently encountered in ovarian cancer (Kupryjanczyk et al., 1993). It seems reasonable to make use of therapeutic strategies that target the p53 pathway of tumour suppression.

For us to be able to properly comprehend pathological apoptosis in the human ovary, it is imperative that we have a grasp of the underlying principles of physiological apoptotic processes in the ovary.

Apoptosis as cell division guardian

Apoptosis is a form of cell death in which a programmed sequence of events leads to the elimination of cells without releasing harmful substances into the surrounding area (Kerr et al., 1972). Apoptosis plays a crucial role in developing and maintaining the health of the body by eliminating old, unnecessary and unhealthy cells. Two main pathways of apoptosis have been elucidated: the death receptor (extrinsic) pathway and the mitochondrial (intrinsic) pathway of apoptosis. The

extrinsic pathway of apoptosis involves binding of ligands to cell surface receptors of the tumour necrosis factor-alpha (TNF- α) superfamily. The death receptor-ligand binding leads to transmission of the death signal through death domains, death effector domains and caspases recruitment domains. The caspase recruitment domains cause the activation of procaspases and adaptor proteins. Procaspases are then cleaved to form active caspases which lead to apoptosis (Hussein, 2005). The intrinsic pathway is regulated by members of the Bcl-2 family. Upon reception of a death signal such as ultra violet rays, gamma radiation oncogenic activation or chemotherapeutic agents by the cell, pro-apoptotic members of the Bcl-2 family are stimulated. This leads to formation of pores on the mitochondrial membrane enabling the release of cytochrome *c* out of mitochondria into the cytoplasm (Hussein et al., 2003). Cytochrome *c* then binds to Apaf 1, alongside dATP to form the apoptosome complex (Gewies, 2003; Spierings et al., 2005). The apoptosome complex cleaves caspase 9, which in turn activates a cascade of events involving executioner caspases resulting in apoptosis (Gupta, 2001; Spierings et al., 2005).

Role of apoptosis in ovarian follicle development and maintenance

The ovary provides a paradigm for programmed cell death due to the cyclic nature of ovarian development and function (Hussein, 2005). Apoptosis is critical for ovarian function, bearing in mind that it regulates the cyclical processes in the female reproductive system. Apoptosis has been shown to be the underlying mechanism of cell death in the ovaries. It has been observed in germ cell loss (germ cell attrition), follicular atresia, corpus luteum regression (luteolysis) and ovarian surface epithelial cells prior to ovulation (Murdoch 1995, Murdoch and McDonnell, 2002; Tilly, 1996; Hussein, 2005).

Apoptosis in germ cell attrition

About seven million oocytes are produced in the ovary during the early life of the human foetus. However, there is a drastic reduction of the number of oocytes to about one third by apoptosis, shortly after birth. The reasons for such a dramatic depletion of the follicular reserve are not yet known. However, Monniaux (2002) proposed that quality control (for the elimination of genetic defects) and a deficit of survival factors from adjacent cells are possible mechanisms. Mutant mice, gene-knock out or transgenic mice have confirmed the involvement of certain molecular effectors in germ cell attrition. Perez et al., (1999), showed that Bax knock-out mice, though normal at birth, increased the number of oocytes during puberty and during their fertile lives. Bcl-2 (B-cell lymphoma

2) transgene mice showed increased folliculogenesis and development of teratomas (Hsu et al., 1996), and knockout experiments also showed loss of oocytes (Ratts et al., 1995). Defects in apoptosis or sustained proliferation of germ cells may lead to germ cell tumours of the ovary.

Follicular atresia

Follicular atresia is the breakdown and resorption of ovarian follicles which occurs prior to ovulation (Santos et al., 2008). Several studies have shown that apoptosis of granulosa cells is the main mode of cell death in follicular atresia (Tilly et al., 1991; Kaipia and Hsueh, 1997; Manabe et al., 2004). Surging Follicle-stimulating hormone (FSH) levels during follicle recruitment enables some antral follicles to evade apoptotic death. A dominant follicle escapes death by secreting oestrogen and inhibin. FSH is thereby, inhibited by inhibin via a negative feedback mechanism. This leads to a negative selection by apoptotic death of the remaining follicles in the group (Hussein, 2005). A positive selection of the dominant follicle is simultaneously enforced by the local secretion of growth factors (McGee and Hsueh, 2000). The dominant follicle is subsequently ovulated. A handful of ligands and their receptors have been shown to be involved in the process; albeit the list of biomolecules continues to expand. They are: TNF- α , TNF-related apoptosis inducing ligand (TRAIL), Fas ligand and APO-3 ligand (Manabe et al., 2008; Kaipia et al., 1996; Quirk et al., 1995). They induce apoptosis via the extrinsic pathway, although the mechanism of TNF- α mediated apoptosis is not known. The fate of the cell is decided by a fine balance between pro-survival and pro-apoptotic molecules. Pro-survival molecules such as insulin-like growth factor-1 (IGF-1), gonadotropins, epidermal growth factor, B-cell lymphoma-extra large (Bcl-xL), Bcl-2, X-linked inhibitor of apoptosis protein (XIAP), Neuronal apoptosis inhibitory protein (NAIP), and integrins are involved in keeping the cell alive, while anti-survival molecules such as, bax, Fas antigens, p53, TNF, Fas/Fas ligand, granzyme-B, GATA-4, IGF favour cell death (Gospodarowicz et al., 1977; Driancourt et al., 1998; Besnard et al., 1996; Matsumoto et al., 1999; Vaskivuo et al., 2001; Amsterdam et al., 2003; Tilly, 2001; Jiang et al., 2003; Hussein, 2005; Caldas et al., 2006; Aoudjit and Vuori, 2012).

Corpus luteum regression (Luteolysis)

Luteolysis is the degeneration of the corpus luteum which occurs at the end of the female reproductive cycle in the absence of pregnancy (Vaskivuo and Taipanainen, 2003). Luteolysis is comprised of both structural and functional processes. The functional process is characterized by an initial drop of progesterone secretion.

On the other hand, the structural process is characterized by changes in the morphology of the cells of the corpus luteum, accompanied by an involution to a smaller mass of whitish connective tissue called the corpus albicans. The corpus albicans often persists in the ovary for several weeks. Data exists to prove unequivocally that apoptosis is the mechanism of luteolysis in humans (Shikone et al., 1996; Vaskivuo et al., 2002). The molecular effectors that have been shown to be mediators of luteolysis include Fas/Fas ligand, prostaglandin F₂- α , endothelins, integrins and interferon- γ (Moeljono et al., 1977; Quirk et al., 1995; Otani et al., 1996; Petroff et al., 2001; Wall et al., 2003).

Apoptosis in ovarian cancer and its therapeutic targets

We have made substantial progress in our understanding of cancer biology and genetics over the past decade. The realization that a malignant phenotype is greatly affected by apoptosis and its related genes is of paramount importance. It is now clear that tumour initiation, transformation or metastases are induced by some oncogenic mutations that disrupt apoptotic mechanisms (Lowe and Lin, 2000). Moreover, it is now well known that most cytotoxic chemotherapy induce apoptosis, suggesting that dysfunctional apoptotic mechanisms significantly contribute to treatment failure. Targeted therapy takes advantage of the fact that apoptotic pathways can be manipulated to induce significant apoptotic cell death by targeting specific genes and proteins that regulate such pathways. Recently, most anticancer agents now in use were developed with the sole purpose of inducing apoptosis that will selectively kill tumour cells. So, targeting apoptosis remains the main focus and safest route towards combating cancer.

Targeted therapy in ovarian cancer

Despite optimal chemotherapeutic and surgical treatment of ovarian cancer patients presenting with advanced disease, 10 to 15% show long-term subsidence. More than 70% of chemotherapeutically treated ovarian cancer cases show resistance, and subsequent relapse to platinum-based drugs and paclitaxel (Copeland et al., 1994; Bartel et al., 2008; Gavalas et al., 2010). This necessitates research into alternative therapeutic strategies against ovarian cancer. Conventional cancer chemotherapy is cytotoxic, and indiscriminately targets rapidly-dividing cells of the body. In a bid to destroy cancerous cells, some normal body cells are killed in the process, resulting in serious side effects (American Cancer Society, 2006). Damages to hair follicular cells, bone marrow cells, gastro-intestinal cells and cells lining the reproductive tract, account for a great proportion of

side effects experienced by chemotherapeutic use. Also, additional limitations of traditional chemotherapy include the palliative and unpredictable responses produced by patients (Arora and Scholar, 2005).

However, targeted therapy depicts a new generation of anti-cancer therapeutics that are designed to ideally interact with a specific molecule, usually a protein molecule, that is believed or shown to have a vital role in tumour progression or growth (Wu et al., 2006; Arora and Scholar, 2005). Researchers have proposed a set of criteria that qualify an ideal molecular target in cancer therapy. They include the following:

1. The molecule should be significantly expressed in vital tissues.
2. It should be reproducibly measured in clinical samples.
3. It should correlate with clinical outcome; yielding a clinical response in a significant amount of patients whose tumours express the target, and shows a trivial response in those whose tumours do not express the target (Ross et al., 2004).

However, side effects have been reported in numerous clinical trials involving small molecule tyrosine kinase inhibitors some of which are as a result of unintended targets. They include fatigue, bowel perforations and severe diarrhea, hypertension, hand-foot syndrome and proteinuria as observed in some vascular endothelial growth factor receptor (VEGFR) and epidermal growth factor receptor (EGFR) inhibitors (Friedlander et al., 2007; Azad et al., 2008; Nimeiri et al., 2008; Biagi et al., 2008).

As opposed to conventional chemotherapy, a hallmark of the action of targeted cancer therapeutics is a higher degree of selective toxicity. This aspect of an anticancer drug may be improved upon by either augmenting the concentration of the therapeutic agent that reaches the tumour tissue or decreasing the amount that reaches normal tissues (Singh et al., 2010). However, synergistic anticancer effects have been achieved by using targeted therapeutic approaches in combination with cytotoxic chemotherapy (Aurora and Scholar, 2005). Various targeted therapeutic strategies have been explored in ovarian cancer management, often used in combination with chemotherapeutic agents for maximal results. Targeted cancer therapeutics can be grouped into two broad categories: small molecule inhibitors and monoclonal antibodies (Wu et al., 2006; Aurora and Scholar, 2005). However, targeted therapy also encompasses RNA inhibition strategies which have been also explored in this manuscript.

Targeting Bcl-2 Family in ovarian cancer and apoptosis

Bcl-2 family of proteins is divided into two types of proteins: those that can induce apoptosis referred to as

pro-apoptotic and those that inhibit apoptosis called anti-apoptotic molecules. The most common protein which is well defined is Bcl-2 which is an anti-apoptotic molecule that exerts its effects by binding to Bax, blocking c-Myc-induced apoptosis, blocking mitochondrial release of cytochrome-c and also inhibiting Apaf-1 interaction with Caspase-9 (Luo et al., 1997).

In the ovaries, Bcl-2 is expressed mainly in healthy ovarian follicles while Bax, a pro-apoptotic molecule, is expressed in the follicles undergoing atresia. Bcl-2 and Bax expressions are markedly influenced by gonadotrophin levels. Elevated gonadotropins tend to inhibit Bax expression while increasing Bcl-2 and Bcl-xL expression, hence promoting the survival of the follicle (Tilly et al., 1995; Sugino et al., 2000). Tilly et al. (1995), analyzing the expression of Bcl-2 family of protein in the immature ovaries of a rat during follicular atresia found the correlation between Bax mRNA which was upregulated and that of Bcl-2 and Bcl-xL down-regulated. Other pro-apoptotic molecules such as Mcl-1, Bax and Bok elicit their apoptotic effects by triggering mitochondrial cytochrome-c release. Cytochrome c binds to Apaf-1, forming the apoptosome and activating the Caspase cascade which leads to apoptotic cell demise (Tilly et al., 1995).

Because of the importance of pro- and anti-apoptotic proteins in deciding if the cell undergoes apoptosis or they survive, they have become targets for many researchers who are eager to restore apoptosis in cancer cells. Ovarian cancer is mainly associated with mutations in some of the genes that might trigger apoptosis and restoring their functions might be alternative therapeutic options. In recent years, several small molecules that aim to target Bcl-2, thereby inhibiting its activity in cancer cells have been identified as possible therapeutic options. One of the recently discovered molecules that act as a selective inhibitor of Bcl-2 is AB-737 and its orally active product AB-263 were shown to inhibit cell growth in eight different ovarian cancer cell lines, although with relatively poor potency. Further tests revealed that ABT-737 increased the sensitivity of several cell lines to carboplatin when ABT-737 was administered after carboplatin (Witham et al., 2007; Jain et al., 2014). In addition, ABT-737 significantly increased carboplatin activity in patients recently treated with chemotherapy (Witham et al., 2007). Co-administration of carboplatin and siRNA directed against Bcl-2 or Bcl-xL resulted in a high growth inhibition in tumour xenographs as compared to carboplatin administration alone (Witham et al., 2007). In recent clinical trials, ABT-737 is able to prolong the survival of recipient mice transplanted with Bcl-2-transduced tumors. It was also found to be functional with co-treatment with chemotherapy.

The second most common molecule which aims to target Bcl-2 is AT101, the importance of this molecule unlike ABT-737 is that it can bind with high affinity to Bcl-xL, Bcl-2 and Mcl-1 and it induces cell apoptosis by activating Bax through a conformational change, trans-

location, and oligomerization that lead to cytochrome c release and effector caspase 3 cleavage (Zerp et al., 2009). Another promising molecule which is on clinical trials phase I and II is Obatoclax (GX15-070), a small-molecule inhibitor of antiapoptotic Bcl-2 proteins which has been reported to trigger cell death via autophagy in ovarian cancer cells (Basit et al., 2013). In another study in a panel of ovarian cancer cell lines, selected for oxaliplatin resistance, Crawford et al. (2011) showed that Obatoclax was able to decrease cell viability irrespective of platinum resistance status. While another molecule HA14-1 is said to inhibit Mcl-1 thereby increasing cell death in ovarian cancer cell lines. Mcl-1RNA interference assisted HA14-1 to induce apoptosis in the absence of chemotherapy (Simonin et al., 2009).

Minimizing expression of Inhibitors of Apoptosis (IAP) as targets for ovarian cancer

The inhibitor of apoptosis proteins (IAPs) constitutes a family of highly conserved group of proteins that are involved in apoptosis, immunity, inflammation cell cycle regulation and migration (Lopez and Meier, 2010). Although, IAP homologs have recently been demonstrated to suppress apoptosis in mammalian cells, their expression and role in human ovarian epithelial cancer and chemotherapy resistance are still unknown or unclear. To date, eight IAPs have been identified: NAIP (BIRC1), c-IAP1 (BIRC2), c-IAP2 (BIRC3), X-linked IAP (XIAP, BIRC4), Survivin (BIRC5), Apollon (BRUCE, BIRC6), Livin/ML-IAP (BIRC7) and IAP-like protein 2 (BIRC8) (Vucic and Fairbrother, 2007). IAPs, inhibit the caspases by binding their conserved BIR domain to the active sites of caspases. This in turn either induces the degradation of active caspases or the separation of caspases from their respective substrates (Wei et al., 2008). Dysregulated IAP expression has been reported in many cancers. Abnormal expression of IAPs, which also correlated with resistance to chemotherapy, has been shown in pancreatic cells (Lopes et al., 2007).

When designing novel drugs for cancers, the IAPs are attractive molecular targets. So far, XIAP has been reported to be the most potent inhibitor of apoptosis among all the IAPs. It effectively inhibits the intrinsic as well as extrinsic pathways of apoptosis by binding and inhibiting upstream caspase-9 and the downstream caspases-3 and -7 (Svane et al., 2004). In normal ovaries, surging FSH in the ovary upregulates XIAP which leads to a suppression of granulosa cell apoptosis and promotes the growth of follicles induced by FSH (Hussein, 2005; Xiao et al., 2001; Scott et al., 2005). So if this is true even during ovarian cancer development, XIAP might provide much better molecular therapeutic target in chemo-resistant ovarian cancer. In recent years, some novel therapy targeting XIAP included short interfering RNA (siRNA) molecules and anti-sense

strategies. In using the antisense approach, Dai et al. (2009) reported that inhibition of XIAP resulted in an improved *in vivo* tumour control by radiotherapy. In another study by Li et al. (2001), they have shown through the use of cisplatin-sensitive and -resistant human ovarian surface epithelial (hOSE) cancer cell lines and adenoviral antisense and sense complementary DNA expression to examine the role of IAP in the regulation of apoptosis in human ovarian cancer cells and chemoresistance. Cisplatin consistently decreased XIAP content and induced apoptosis in the cisplatin-sensitive, but not cisplatin-resistant, cells.

Resistance to cisplatin in ovarian cancer, arises from the dysregulation of tumour suppressors and survival signals. Piceatannol which is a natural metabolite of the stilbene resveratrol found in grapes and is converted from parent compound by the enzyme CyP1BA/p450. Piceatannol was found to enhance p53-mediated expression of the pro-apoptotic proteins NOXA, and also increased XIAP degradation via the ubiquitin ligases and enhance caspase-3 activation (Farrand et al., 2013). Another recently identified molecule that seemed to suppress the expression of XIAP is LBW242, which is a small molecule that mimics SMAC/DIABLO this molecule was shown to activate apoptosis in both primary ovarian cancer cells and ovarian cancer cell lines by activating caspase-8 (Eschenburg et al., 2012; Petrucci et al., 2012). LBW242 also sensitizes ovarian cancer cells to the antitumor effects of TRAIL and commonly used anticancer drugs. Birinapant (TL32711), a synthetic small molecule and peptidomimetic of second mitochondrial-derived activator of caspases (SMAC) and inhibitor of IAP family of proteins, has demonstrated strong correlation between drug exposure, target coverage and apoptosis induction in tumours at well-tolerated doses as well as promising anti-tumour activity in patients (Nguyen et al., 2013).

Therapeutic potential of TNF family members

Tumor necrosis factor alpha (TNF- α), is the prototypic ligand of the TNF superfamily. It is a pleiotropic molecule that plays a key role in inflammation, apoptosis and development of the immune system. In normal ovarian development, the expression of some TNF family members such as FasL/Fas is highly influenced by gonadotrophin levels. Surging gonadotrophin levels result to a decreased expression of Fas/FasL, thereby promoting follicular survival. However, decreased gonadotropin levels result in an increased expression of Fas/FasL, leading to follicular atresia (Jiang et al., 2003). TNF- α is highly expressed in ovarian cancer. TNF- α modulates the expression of CD44 in normal T lymphocytes and CD44 is involved in the carcinogenesis and metastasis of ovarian cancer (Muthukumaran et al., 2006). TNF family members show both pro-survival and

pro-apoptotic functions, depending on the type of receptors that are activated. TNF- α triggers apoptosis by activating caspases (Wang et al., 2008); on the other hand, TNF- α is able to aid the survival of granulosa cells by upregulating the expression of and XIAP through the NF κ B system (Jiang et al., 2003). TRAIL is another TNF family member that has been shown to induce apoptosis in tumour cells, but not in normal cells, owing to the presence of TRAIL decoy receptors which competitively inhibit the binding of TRAIL ligands to their cognate receptors (Sheridan et al., 1997; Pan et al., 1997). TRAIL and its receptors are expressed in growing, atretic and antral ovarian follicles (Bobe and Goetz, 2001).

Wt p53: the genomic guardian target

TP53 is a gene that encodes the tumour suppressor protein, p53, which contains a DNA-binding domain, a transcriptional activation domain and an oligomerisation domain (Harms and Chen, 2006). P53 responds to various cellular stresses to regulate the expression of target genes, thereby inducing cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism (Bates and Vousden, 1999). Mutations in this gene are associated with a variety of human cancers (Toledo and Wahl, 2006). The effects of p53 are mainly as a result of its activity as a transcription factor. It transcriptionally activates pro-apoptotic genes such as Bax, PUMA, NOXA and the growth arrest gene p21. It represses the transcription of anti-apoptotic genes such as Bcl-2 and Bcl-xL, MDM2 (Barak et al., 1994; Wu et al., 2001; Ohtani et al., 2001). p53 is expressed in apoptotic granulosa cells of atretic follicles, suggesting a possible role of p53 in follicular atresia (Kim et al., 2000). One of the ways by which Gonadotropins inhibit apoptosis is by repressing a host of pro-apoptotic genes including p53 gene expression (Tilly et al., 1995). It has been shown that mutant p53 can be refolded to the wild type conformation by an active compound, MQ, which is converted from the inactive form, APR-246. This leads to p53-dependent apoptosis (von Euler et al., 2014).

In addition to reactivating p53 in ovarian cancer, APR-246 also decreases the intracellular levels of glutathione in a dose-dependent fashion (von Euler et al., 2014). The targeting of both p53 and glutathione probably accounts for the strong synergistic effects of APR-246 and platinum-based drugs in ovarian cancer cells (von Euler et al., 2014). Ad5CMV-p53 is a recombinant adenoviral vector that encodes TP53. The co-administration of Ad5CMV-p53 intraperitoneally with chemotherapy using gemcitabine, showed synergistic effects in the treatment of recurrent disease after multiple cycles of therapy (Wen et al., 2003). In another study, it was shown that Ad5CMVp53 showed a marked clinical activity when used in combination with platinum-based chemotherapy to treat recurrent ovarian carcinoma (Buller et al., 2002). Cytoplasmic-Domain-of-Band-3-

Protein-(cdb3), which is a synthetic p53 binding protein-derived peptide interacts with the core domain of p53, and upregulates the transactivation activity of p53 (Samuels-Lev et al., 2001). A fluorescently tagged Cytoplasmic-Domain-of-Band-3-Protein-(cdb3) was shown to improve the proper native folding of p53Arg273His contact and p53Arg175His mutants; induce p53-mediated transcriptional activation of p21, GADD45 and MDM2 and induce apoptosis. Even though the wild-type bearing cells did not undergo apoptosis after treatment with Cytoplasmic-Domain-of-Band-3-Protein-(cdb3) alone, the cells showed enhanced sensitivity to apoptosis induced by infra-red radiation (Issaeva et al., 2003).

A small synthetic molecule of the styrylquinazoline family known as Cp-31398 has been identified for its ability to stabilize p53 against thermal denaturation *in vitro*. Treatment with Cp-31398 hinders p53 ubiquitination and degradation leading to an enhanced cell surface exposure of DR5 and an activation of the intrinsic bax/mitochondrial/caspase-9 pathway, culminating in apoptosis (Vecil et al., 2003). Because of the critical inhibitory role of MDM2 and RBBP6 on p53 (Li et al., 2007), blocking the interaction of RBBP6, MDM2 and p53 has been proposed as a potential cancer therapeutic strategy (Moela et al., 2014). Nutlin-2 is a small-molecular-weight inhibitor that fits into the pocket where wild-type TP53 binds to MDM, a molecule required for the rapid degradation of TP53 through the ubiquitin-proteasome pathway (Wang et al., 2012). Inhibition of the MDM-TP53 interaction results in the increased expression of wild-type TP53, inhibiting tumor growth and inducing apoptosis. In a study by Moela et al. (2014) have shown that silencing breast cancer cell lines with RBBP6 siRNA followed by treatment with camptothecin also sensitized cancer cells to apoptosis induced cell death which serves as a promising natural cell biology pathway.

Other apoptotic molecular targets for ovarian cancer cells

Interferons (IFN)

They include IFN- α , IFN- γ , IFN- β and IFN- δ . IFN- γ is a cytokine that has potent immunomodulatory, antiviral, and antiproliferative that has anticancer activity. IFN- γ directly inhibits human tumor cell growth and induces apoptosis (Clemens, 2003). Interferons sensitize cells to apoptosis-inducing genes and proteins in apoptotic pathways (O'Connell et al., 2000; Lissat et al., 2007). IFN- δ inhibits Fas expression in the corpus luteum, thereby inducing apoptosis in bovine luteal cells (Komatsu et al., 2003). This is as a result of anti-apoptotic Fas effects in the corpus luteum. IFN- γ has been shown to induce apoptosis in luteal cells and ovarian cancer cells both *in vivo* and *in vitro* (Petroff et al.,

2001; Wall et al., 2003). In ovarian cancer, cell lines and xenografts in nude mice IFN was found to induce apoptosis and inhibit proliferation of cancer cells (Wall et al., 2003). IFN- γ might be a useful biological treatment of human epithelial ovarian cancer if sustained levels of this cytokine could be achieved within the peritoneum by improved protein or gene delivery strategies.

GATA-4

GATA-4 is a zinc finger transcription factor that induces cell proliferation. GATA-4 expression during foetal development is expressed in granulosa cells of primary and antral follicles (Orkin, 1992). GATA-4 expression has been shown to correlate with active granulosa cell proliferation in adult human ovaries; therefore possibly functioning as a pro-survival molecule in the ovary (Vaskivuo et al., 2001). In adult mouse ovaries, GATA-4 down-regulation correlates with follicular atresia. GATA-4 expression has been shown to be stimulated in gonadal cell lines by exogenous gonadotropins. However, the exact mechanism of GATA-4 mediated cell proliferation is not known (Heikinheimo et al., 1997; Vaskivuo et al., 2001). Targeting GATA-4 using RNA interference mechanisms in combination with standard chemotherapy may be useful in treating ovarian cancer.

Prostaglandin F2- α receptor

It shows pro-apoptotic effects in the ovary. It is expressed in the corpus luteum, thecal cells and interstitial cells. It is however, absent in granulosa cells at all stages of follicular development and also in oocytes. It is involved in apoptosis in the corpus luteum of during luteolysis. It upregulates endothelin-1 expression which plays a role in luteolysis (Moeliono et al., 1977; Orlicky et al., 1992; Girsh et al., 1996).

Endothelins

These are protein molecules that function in vasoconstriction, mitogenesis and steroid production (Luscher and Barton, 2000). FSH is known to exert its effects by stimulating cyclic-AMP action. FSH regulates Endothelin-1 levels in a dose-dependent manner (Otani et al., 1996). Endothelins abrogate the action of cyclic AMP mediated by FSH. ET-1 is over-expressed in the corpus luteum during luteolysis and is thought to have pro-apoptotic effects in the corpus luteum (Otani et al., 1996).

Integrins

that connect the cell to the cytoskeleton, and are able to influence cell survival and cell death (Aoudjit and Vuori, 2012). They function in cell proliferation via signal trans-

duction pathways by activating protein kinases (Giancotti and Ruoslahti, 1999). Integrins are expressed in primordial follicular cell surfaces, aiding their adhesion to the extracellular matrix. Integrins are weakly expressed in atretic tertiary follicles and absent in atretic primary and secondary follicles. Granulosa cells that lack the expression of integrins were shown to be the only ones that undergo apoptosis (Giebel et al., 1996). Ovarian tumour growth and angiogenesis were shown to be inhibited when volociximab, a chimeric monoclonal antibody was directed against $\alpha 5\beta 1$ -integrin (Sawada et al., 2008). Intetumumab (CNT095) is a humanized anti-integrin monoclonal antibody that targets and binds with high affinity (Kd, approximately 1–24 nmol/L), to cells expressing αv integrin. Intetumumab has been shown to inhibit cell adhesion, invasion, migration, and proliferation of tumour and endothelial cells in vitro. Intetumumab also reduced tumour metastasis in vivo in nude mice with human breast cancer xenografts by inactivating the focal adhesion kinase (FAK) and the docking protein paxillin (Ning et al., 2010). Intra-peritoneal administration Etaracizumab, a humanized antibody, in SKOV3ip1 and HeyA8 mouse model ovarian cancer tumours targeting the $\alpha v\beta 3$ integrin receptor decreased tumour burden by 36 and 48% respectively (Landen et al., 2008). However, its role in ovarian cancer patients is still to be exploited in clinical trials. Cilengitide, an $\alpha v\beta 3$ and $\alpha v\beta 5$ integrin inhibitor, is a cyclic RGD containing pentapeptide. Perry et al. (2013), using breast cancer cell lines (MCF-7 cells and then MDA-MB-231) treated with Cilengitide has shown that it was able to induce apoptosis and arrest cell proliferation. In another study, a combined approach with cilengitide and radiotherapy in breast cancer cells showed higher efficacy than either treatments alone (Lautenschlaeger et al., 2013). This study together with others have actually supported the idea that cilengitide in combination with radiation can be useful tool against ovarian cancer associated with integrins.

Insulin-like growth factor (IGF)

IGFs are proteins with high sequence similarity to insulin that stimulate mitosis; regulating cell proliferation, differentiation and apoptosis. IGF binding proteins regulate the activity of IGFs by modulating IGF binding to their cognate receptors (Werner et al., 2008). IGF-1 functions in the ovary by potentiating the action of gonadotrophic hormones. IGF-1 stimulates and sustains signals that lead to cell proliferation and inhibition of apoptosis by activation of the P13K/AKT pathway (Zheng et al., 2002). A high IGF-1 expression in mice follicles suggests that it plays an important role in follicle development. The roles of IGF binding proteins may differ; while IGFBP-4 is highly expressed in atretic follicles, IGFBP-5 is up-regulated in healthy primary and secondary follicles (Besnard et al., 1996). IGF over-

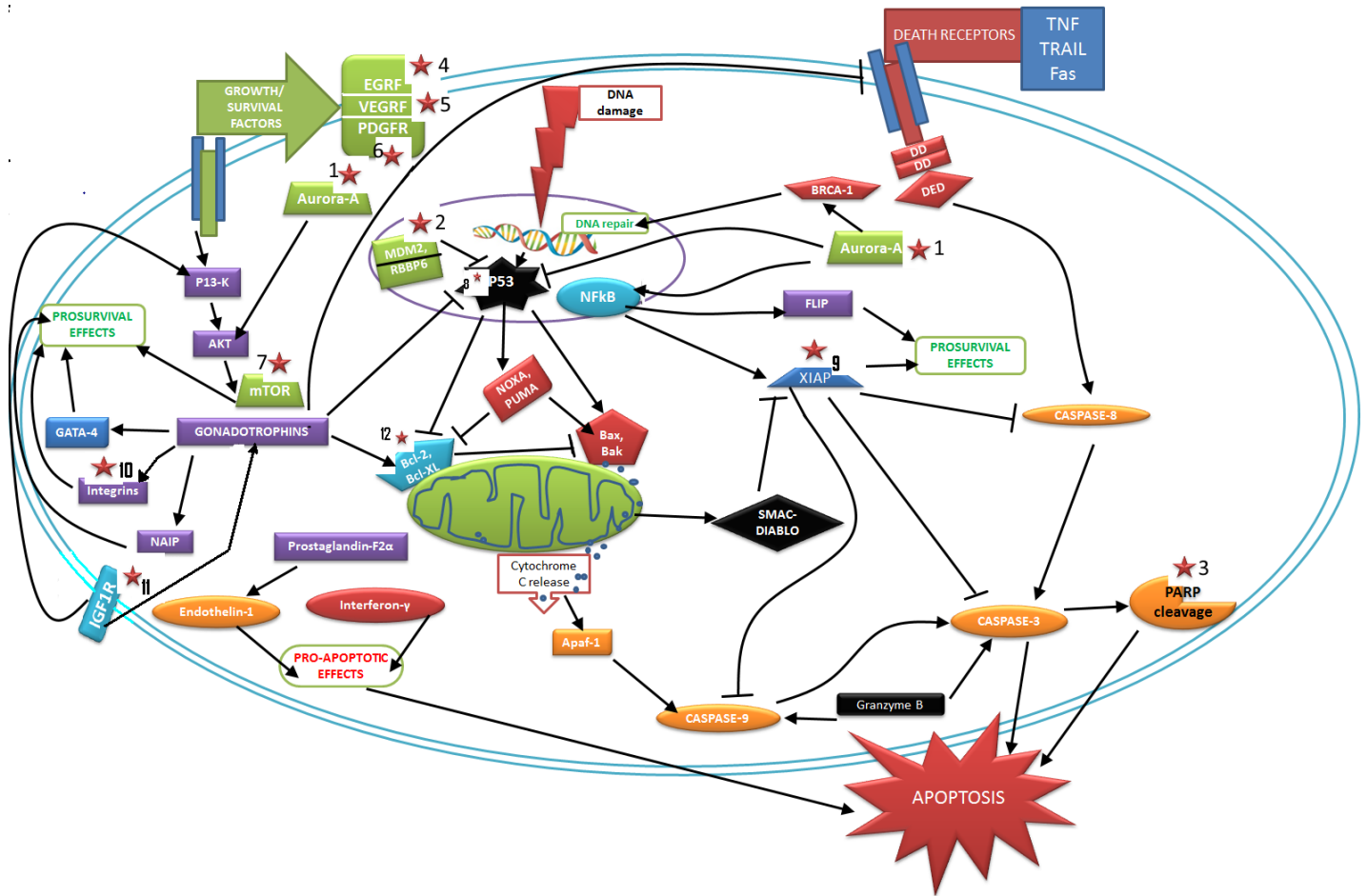


Figure 1. A schematic mechanism showing apoptosis in ovarian cancer and some current targeted therapeutic approaches.

expression correlates with poor disease prognosis in some cases of ovarian carcinomas (Brokaw et al., 2007).

Small molecule inhibitors impede IGF-IR activation by binding to the ATP-binding pocket of the receptor. Insulin receptor signaling can be attenuated by most tyrosine kinase inhibitors. Despite this side effect due to lack of specificity, they showed activity in preclinical models and some are being evaluated in clinical trials. A potent insulin-like growth factor type 1 receptor (IGF-1R) inhibitor, NVP-AEW541, was shown to sensitize ovarian cancer cells to the effect of cisplatin (Beauchamp et al., 2010). In addition to apoptosis induction, it was found that AKT activation was also decreased by NVP-AEW541 (Beauchamp, et al., 2010) in two human epithelial ovarian cancer cell lines, namely, OVCAR-3 and OVCAR-4. Another molecule exploited is, BMS-536924 which is a potent small molecule inhibitor of IGF-IR, which shows antitumor activity in multiple tumour models. It has been shown to induce apoptosis in ovarian cancer cells by activating the cleavage of Poly ADP Ribose Polymerase (PARP) (Beauchamp et al., 2009). This shows that co-administration of BMS-536924 and a PARP inhibitor might be an effective strategy to curb resistance in

ovarian cancer. In another study by Beltran et al. (2014) using AMG 479, a fully human monoclonal antibody against IGF-1R, they reported that AMG479 served as second line therapy in patients with recurrent platinum-sensitive ovarian cancer by blocking the binding of IGF1 and IGF2. There is a possibility that these agents might be more potent anticancer drugs since insulin receptor present on malignant cells may have an important role as well in carcinogenesis (Figure 1.)

Central to this mechanism, is the activity of Gonadotrophins and other upregulated proteins such as Aurora A, PARP, mammalian target of rapamycin (mTOR) and growth factors such as EGFR, VEGFR, and Platelet-derived growth factor receptors (PDGFR) which have been exploited as molecular targets in various types of epithelial ovarian cancer and are at various phases in clinical trials. The numbered red stars represent various molecular targets that have been explored in ovarian cancer. Aurora A is a potent prosurvival molecule that exerts its effects by activating AKT, promoting DNA repair by activating BRCA1, blocking p53 activity, and activating the transcription factor NFκB. Growth factor receptors [EGFR (4), VEGFR (5), and PDGFR (6)] which exert

Table 1. A table showing small molecule inhibitors/ monoclonal antibodies and their molecular targets in ovarian cancer.

Small molecule drug (generic name)	Molecular target	Clinical trial phase	References
Tozasertib (MK-0457)	Aurora kinase	Phase I	Sun et al. (2007), Lin et al. (2008) and Traynor et al. (2011)
Nultin-2	MDM2	Phase I	Wang et al. (2012)
Olaparib	PARP (Poly-ADP ribose)	Phase I	Fong et al. (2008)
Erlonitib and gefitinib	EGFR (epidermal growth factor receptor)	Phase II (gefinitib), Phase III (erlonitib)	Schilder et al. (2005) and Vergote et al. (2013)
BMS-690514	VEGFR (vasculo-endothelial growth factor receptor)	Not yet entered clinical trials for ovarian cancer	Becker et al. (2013)
Imatinib mesylate (STI57)	PDGFR (Platelet-derived growth factor receptor)	Phase II	Alberts et al. (2007)
Everolimus (RAD001)	mTOR	Phase I	Mabuchi et al. (2007) and Okamoto et al. (2010)
APR-246	P53	Phase II	Von Euler et al. (2014)
LBW242	XIAP	Phase I	Eschenburg et al. (2012) and Petrucci et al. (2012)
Nil	Integrin	Not entered clinical trials for ovarian cancer	Landen et al. (2008)
BMS-536924, NVP-AEW541, Ganitumab (AMG-479)	IGFR1	Not entered clinical trials for ovarian cancer Phase I	Beauchamp et al. (2009, 2008) and Beauchamp et al. (2010)
ABT-737	Bcl-2/Bcl-XI	Phase I	Witham et al. (2007) and Jain et al. (2014)

prosurvival effects via the P13K/AKT pathway have been targeted in ovarian cancer. XIAP, which can be upregulated by growth signals from NFkB is the most potent known inhibitor of caspases and has also been targeted using small molecules. Integrins such as $\alpha 5\beta 1$ -integrin exert prosurvival effects by activating protein kinases. IGF-1 functions in the ovary by potentiating the action of gonadotrophic hormones. IGF-1 stimulates and sustains signals that lead to cell proliferation and inhibition of apoptosis by activation of the P13K/AKT pathway. MDM2 which interacts with RBBP6 to promote p53 ubiquitination has been targeted as well with Nultin-2 (Table 1).

Tyrosine kinases inhibitors

Tyrosine kinases are molecules that play crucial roles in signal transduction, culminating their

effects by regulating gene transcription within the nucleus. They function by transferring γ -phosphate groups from adenosine triphosphate to the hydroxyl group of protein molecules responsible for signal transduction (Schlessinger, 2000). A major event that activates tumour proliferation is the phosphorylation of signal transduction molecules. The most essential cellular processes such as the cell cycle, cell division, differentiation, motility and apoptosis or cell survival are under scrupulous regulation of tyrosine kinases (Prenzel et al., 2001; Slichenmyer and Fry, 2001). A dysfunction in tyrosine kinases pre-dispose signal transduction molecules to sustained phosphorylation; hence abnormal cell proliferation. Tyrosine kinases have been found over-expressed or mutated in several types of tumours in humans including ovarian tumours (Levitzki and Gazit, 1995; Blume-Jensen and Hunter, 2001; Wiener et al., 2003) making them good targets for cancer therapy. Small

molecules have been developed to target tyrosine kinases in ovarian cancer.

Angiogenesis inhibitors

One of the key features leading to metastasis and invasion of normal tissues by cancerous cells is angiogenesis (the formation of new blood vessels). Poor prognosis in ovarian cancer has been confirmed to be associated with increased expression of the vascular endothelial growth factor (VEGF) which has functions such as angiogenesis, mitogenesis, improvement of vascular permeability, and endothelial cell survival (Folkman, 1997; Hartenbach et al., 1997). Small molecule tyrosine kinase inhibitors have been developed to target the VEGF ligand and the VEGF receptor in ovarian cancer thereby slowing angiogenesis and improving upon prognosis of disease (Friedlander et al., 2007; Campos et al.,

2008; Hirte et al., 2008; Becker et al., 2013).

Inhibitors of the epidermal growth factor receptor (EGFR)

EGFR is a transmembrane tyrosine kinase protein receptor that is involved in cell proliferation, survival and differentiation (Herbst, 2004). About 70% of ovarian cancers show upregulated expression in EGFR, making it an attractive target for the treatment of ovarian carcinomas. Over-expression has been shown to correlate with chemoresistance and poor prognosis. Increased cell proliferation, angiogenesis and reduced apoptosis are attributed to over-expression of EGFR (Bartlett et al., 1996; Fischer-Colbrie et al., 1997). Tyrosine kinase inhibitors such as erlotinib and gefitinib have been successfully directed against the EGFR (Sirotnak et al., 2000; Sirotnak, 2003). Phase 2 clinical trials with gefitinib in patients with advanced recurrent ovarian carcinomas showed very little activity, although the drug was well tolerated (Schilder et al., 2005). Phase 3 trials with erlotinib, however did not show any significant improvement in activity (Vergote et al., 2013).

Aurora kinase inhibitors

Aurora A is a serine-threonine kinase that is required for many essential cellular functions such as mitosis, spindle formation and centromere separation (Bischoff et al., 1998; Campos and Gosh, 2010). An over-expression of Aurora A as well as amplification of its gene location have been frequently noted in human tumours, including in ovarian carcinomas (Bischoff et al., 1998; Zhou et al., 1998; Lingle et al., 1998; Landen et al., 2007). Aurora A has been shown to inhibit paclitaxel and cisplatin – mediated apoptosis in ovarian cancer cells (Yang et al., 2006; Anand et al., 2003). Aurora kinase inhibition with a small molecule MK-0457 in combination with chemotherapy (docetaxel) has shown significant reduction in tumour growth and cell proliferation in HeyA8 and SKOV3ip1 ovarian cancer cell lines (Sun et al., 2007; Lin et al., 2008; Traynor et al., 2011).

Poly ADP Ribose Polymerase (PARP) inhibitors

BRCA1 and BRCA2 play an important role in DNA double strand break repairs and maintaining genomic stability (Lord and Ashworth, 2008). BRCA ovarian cancer patients show an impaired ability to repair damaged DNA. Mutations in these genes account for 5 to 10% of all ovarian cases. BRCA genes represent the most important risk factor in ovarian cancer with lifetime risks of between 40 to 50% and 10 to 20% for BRCA1 and BRCA2 respectively (Cannistra, 2004; Lord and Ashworth

2008). More than 50% of high grade serous ovarian carcinomas show loss of function of BRCA genes, either by genetic or epigenetic causes (Press et al., 2008). Poly ADP Ribose Polymerase (PARP) is a nuclear enzyme involved in the DNA single strand break repair (Tutt et al., 2005). It is activated in DNA damage and its inhibition results in DNA single strand breaks which may result in double strand breaks. BRCA1 and BRCA 2 patients show high sensitivity to DNA-damaging chemotherapeutics and have also shown immense responsiveness to PARP inhibitors (Bryant et al., 2005; Tutt et al., 2005). Although the mechanism is not fully understood, Lord and Ashworth (2008) suggest that an excessive amount of DNA single strand breaks with subsequent double strand breaks leads to high irreparable genomic instability and hence, cell death. Olaparib is a small molecule PARP inhibitor that has been used and has shown efficacy in BRCA1 ovarian cancer patients and has passed the first phase of clinical trials (Fong et al., 2008).

Platelet-derived growth factor (PDGF) receptor inhibitors

PDGF is involved in cellular growth, survival, differentiation, vascular permeability, cellular migration and healing of wounds (Schmitt and Matei, 2008). Between 50 and 80% of ovarian cancers show activation of the PDGF receptor which is involved in neoplastic transformation (Heinrich et al., 2003; Apte et al., 2004). PDGF receptor activation is as a result of mutations, genetic amplification or chromosomal rearrangements (Carroll et al., 1996; Heinrich et al., 2003). Imatinib mesylate (STI57) is a small molecule that has been used to target PDGF receptor. A significant induction of apoptosis and reduction in tumour weight was observed in this study when STI571 was used in combination with taxol. However, STI571 alone did not cause any significant effects (Apte et al., 2004). Imatinib is a small molecule PDGF receptor inhibitor that has passed phase II clinical trials in ovarian cancer patients whose tumours express the PDGF receptor (Alberts et al., 2007).

MTOR inhibitors

PTEN is a lipid phosphatase that is involved in G1 cell cycle arrest and apoptosis through the AKT /PI3K /mTOR pathway, and has been shown to be mutated, deleted or inactivated in gynaecologic tumours (Sansal and Sellers, 2004; Jiang and Liu, 2008; Campos and Gosh, 2009). The mTOR pathway is a key regulator of cell growth, proliferation and programmed cell death (Campos and Gosh, 2009). Inhibitors of mTOR such as everolimus (RAD001) have been shown to inhibit angiogenesis, tumour proliferation and ascites formation in vivo and in vitro using OVCA10 and SKOV-3 ovarian cancer cells. It is also improved upon cisplatin-mediated apoptosis. These

suggest a promising role of mTOR inhibitors to treat ovarian tumours (Gera et al., 2004; Mabuchi et al., 2007; Okamoto et al., 2010).

CONCLUSION

Just as in other tumours in humans, a detailed understanding of the molecular abnormalities in the various types of ovarian tumours seems to be the only means to maximize the positive therapeutic effects and better prognosis derived from targeted therapy. Similar genetic and histopathological characteristics exist in tumours of the same sub-type (Gilks, 2009).

However, our comprehension of genetic and molecular aberrations including the mechanisms of oncogenic transformation seems to be at its infancy; given that even with targeted therapeutic treatments in combination with chemotherapy, a high proportion of patients still develop recurrent disease and subsequently, death. Apoptosis remains the most important mechanism of cell death in cancer therapy. However, for apoptosis to be selectively induced as a therapeutic measure, the ideal targets need to be found. A diligent search of other molecular targets and an improved understanding of the pathways involved are essential for us to maximize the benefits of targeted therapy.

The genetic and molecular characteristics of ovarian tumours are not the same, even in the same ovarian tumour subtype. The more understanding we achieve, the better the prognosis of ovarian cancer, and the more individualized targeted therapy would become.

Conflict of interest

The authors declare that they have no interest.

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Full Length Research Paper

Anti-ulcer activity of *Rhus coriaria* in indomethacin and water immersion restraint induced gastric ulcer in experimental rats

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The anti-ulcer activity of hydro alcoholic extract of *Rhus coriaria* Linn (HAERC) was investigated in indomethacin and water immersion-induced restraint gastric ulcer in wistar rats. The assessment was carried out by using ulcer index, ulcer score and histopathological studies of the specimens. HAERC at doses of 145 and 248 mg/kg given orally produced significant inhibition of the gastric lesions induced by indomethacin and water immersion restraint method, and the results were comparable to the standard treatment regime. We observed that *R. coriaria* Linn extract exhibits significant anti ulcer activity and thus supports the Unani claims about the drug.

Key words: Rhuscoriaria, indomethacin, water immersion induced ulcer model, ulcer index, postsumaq.

INTRODUCTION

Peptic ulcer disease (PUD) is one of the most common gastro intestinal disorders, which causes a high rate of morbidity. An estimated 15,000 deaths occur each year because of PUD. The prevalence of duodenal ulcer is dominant in western population whereas gastric ulcer is more frequent in most Asian countries (de Sousa Falcão et al., 2008). The lifetime prevalence of peptic ulcer disease is 5 to 10% with about equal prevalence in men and women. The incidence of ulcer increases with age because of excessive use of non-steroidal anti-inflammatory drugs (NSAIDs) and the reduction in the tissue prostaglandins (Anne and Allison, 2003).

In India, PUD is common and the Indian pharmaceutical industries share 6.2 billion rupee and occupy 4.3% of the market share in consuming the

antacids and antiulcer drugs (Calam and Baron, 2001). Peptic ulcer which is usually an asymptomatic gastrointestinal disorder defined as a breach in the mucosa of the alimentary tract, which extends through the muscularis mucosa into the submucosa or deeper. Peptic ulcer disease commonly occurs when the linings of stomach or proximal duodenum are corroded by the acid-peptic juices which are secreted by the stomach cells (Humes 2001; Ledingham and GWarrell, 2000). Peptic ulcer is caused by *Helicobacter pylori* infection, long term and high doses of drugs such as NSAIDs, diseases like Zollinger- Ellison syndrome, other factors such as smoking; emotional stress and excessive alcohol consumption also may contribute. In Unani Medicine, gastric ulcer is known as Qarah-e-Medah.

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Unani scholars mentioned its causes as, Khilte Haad (hot and irritant humour), Fuzlat (waste products), intake of hot and spicy foods, excessive intake of rotten food, alcohol and hard fibrous diet, desensitization of internal surface of stomach which causes excessive gastric secretions, chronic gastritis and indigestion, prolonged stress and strains and unabsorbed gastric secretions. The modern approach to control gastric ulceration is to inhibit gastric acid secretion, to promote gastro protection, to block apoptosis and to stimulate epithelial cell proliferation for effective healing (Bandyopadhyay et al., 2000). Hence, conventional medicine treats peptic ulcer by proton pump inhibitors (PPI), H₂-receptor antagonist, antacids and antibiotics for *H. pylori*.

However, there are reports of adverse effects and relapse in the long run (Raju et al., 2009) that lead people to find the alternative medications. Furthermore, many of these drugs do not fulfill all the beneficial necessities (Dharmani et al., 2005). The clinical evaluation of these drugs showed development of tolerance and incidence of relapse, and side effects that make their efficacy debatable. This has been the rationale for the development of new, safe antiulcer drugs. Herbal drugs can provide lead for the development of such antiulcer drugs because these drugs are considered safer in view of their natural ingredients. In recent times, focus on plant research has increased all over the world and a large body of evidence has been collected to show immense potential of medicinal plants used in various traditional systems of medicine. More than 13,000 plants have been studied during the last few years (Dahnukar et al., 2000).

Unani physicians in the treatment of gastritis, gastric ulcer and associated disorders due to its stomachic, astringent, desiccant, styptic, sedative and coolant activities (Ghani, 2011) also use PostSumaq (Fruit rind of *Rhus coriaria* Linn.) frequently. However, there is no scientific report regarding its efficacy in PUD. Therefore, the present study was carried out to examine the effect of PostSumaq in gastric ulcer on animal model.

MATERIALS AND METHODS

Institutional Animal Ethics Committee (IAEC) of National Institute of Unani Medicine (NIUM) approved the present study. The test drug Sumaq (*R. coriaria* Linn) was procured from local market of Bangalore, and was identified by Dr. H.B. Singh, Chief Scientist and Head of National Institute of Science Communication and Information Resources (NISCAIR) New Delhi, vide Reference No. NISCAIR/RHMD 2030/38.

Preparation and dose of the test drug

The fruits of test drug were dried in shade, the *Post* (rind) was peeled off, and its therapeutic dose (5 gm) as in Unani medicine was used to calculate the dose for experiment (Freirich et al., 1966). Thus, dose was found to be 580 mg/kg. Since, the test drug was studied at two different doses; therefore a second dose was also calculated by the method of Miller and Tainter (1944) and was found to be 990 mg/kg. As the hydro alcoholic extract was used for

the study, the dose of the extract was calculated with reference to the dose of crude drug after obtaining the 25% yield percentage of extract. The hydro alcoholic extract of the drug was used in the dose of 145 and 248 mg/kg. Standard drug, omeprazole (Manufactured in India by Dr. Reddys Laboratories Ltd. Village Manuja Thana) was used in the dose of 20 mg/kg.

Animals

The study was carried out in healthy wistar rats of either sex, weighing 150 to 250 gm. The animals were procured from Biogen Laboratory Animal Facility (Reg. No. 971/bc/06/CPCSEA), a registered breeder in Bangalore. They were acclimatized to the laboratory condition for 7 days before the experimental studies. The rats were housed in polypropylene cages under controlled conditions of light (12/12) and temperature (23±2°C) under strict hygienic conditions. The animals were given Standard food pellets (Hindustan Lever Ltd.) and tap water ad libitum.

Induction of gastric ulcer

This test was carried out by the method described by Vogel (Vogel, 2002) with minor modification in the treatment schedule. The animals were divided into 8 groups of 6 animals each. The animals in group I were administered with distilled water throughout study and served as Plain control, and after 36 h they were sacrificed while the animals in group II (after 24 h of fasting) were treated with indomethacin 20 mg/kg, once daily, orally for 5 days and served as negative control.

The animals in group III, IV and V were treated with standard drug omeprazole and hydro alcoholic extract of test drug in doses 20, 145 and 248 mg/kg, respectively, and served as pre-treated standard, pre-treated test group A and pre-treated test group B, respectively. These treatments were carried out for five days; however, on the 6th day after 24 h of fasting ulcer was induced by the administration of indomethacin in the dose of 20 mg/kg, for the next five days. Food was withdrawn for two hours after Indomethacin administration. On the 5th day after 12 h of fasting, the animals were treated with the last dose of indomethacin and after five hours of administration of indomethacin, the animals including negative control were sacrificed. While in post treated standard and test groups, the animals were first kept on fasting for 24 h and ulcer induced by the administration of Indomethacin in the dose 20 mg/kg, daily for 5 days, thereafter the animals were treated with standard and test drug for the next 5 days in the same dose and same manner as described above. On the 6th day after 12 h of fasting, the animals were sacrificed.

The water immersion restraint induced gastric ulcer was done by the method of Hayaso and Takeuchi (Hayaso and Takeuchi, 1986). The animals in this model were also divided into 8 groups of 6 animals each. The animals in Group I and II were treated with distilled water and were serve as plain control and negative control, respectively. While the animals in Group III, IV and V were treated with standard drug, and hydro alcoholic extracts of the test drug in doses 20, 145 and 248 mg/kg were served as pre-treated standard, pre-treated test group A and pre-treated test group B, respectively. All the animals were treated in this way once daily for 5 days. They had free access to food and water during the treatment period. However, on the 4th day they were kept on fasting for 12 h with water ad libitum. On the 5th day, 12 h fasted rats were treated routinely and after one hour of treatment, animals in Group I were sacrificed while in rest of the groups, ulcer was induced by water immersion restraint method. The animals in Group VI, VII and VIII were also subjected to gastric ulceration in the same manner as mentioned above. After one hour of ulcer induction, animals were treated with standard and test drug and served as

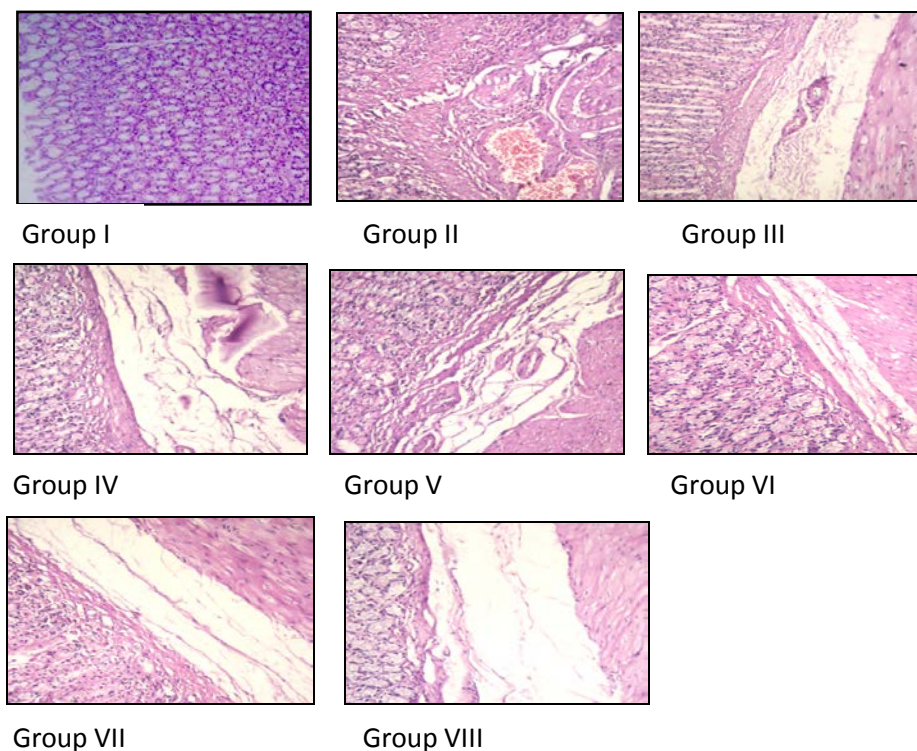


Figure 1. Histopathological slides of different groups (Indomethacin ulcer model): group I (Normal mucosa); group II (Congestion, necrosis, inflammation and ulceration); Group III: (Oedema, necrosis, inflammation and ulcer); Group IV: (Oedema, necrosis and ulceration); Group V: (Inflammatory changes); Group VI: (Inflammatory changes); Group VII: (Oedema, necrosis, inflammation and ulceration); Group VIII: (Inflammation and ulceration).

post-treated standard group (VI) and post-treated test group A and group B (VII and VIII), respectively.

All the animals were treated in this manner orally, once daily for five days. On the 5th day, 12 h fasted animals were treated routinely and after one hour of treatment, they were sacrificed. In all the above methods, the animals were sacrificed under Theopentone anesthesia (40 mg/kg, IP). Stomach was removed from the body and opened along with the greater curvature, washed with fresh water and spread on cardboard with the mucous surface upwards. The mucosal surface was examined for ulceration with the help of magnifying lens (10 fold magnification) and scored by the method of (Brzozowski et al., 1998; Haqeeq et al., 2013; Haqeeq et al., 2013).

Assessment of extent of ulceration

The parameters viz. ulcer score, ulcer index and reduction percentage in ulcer were taken to assess the anti ulcerogenic effect. Histopathological studies were also carried out to determine the nature and amount of damage and the improvement after treatment (Figures 1 and 2).

Statistical analysis

The observations in various groups were expressed as Mean \pm SEM. The ulcer score and index of various groups were compared with plain control group. The group comparison was analyzed by using ANOVA one way with Kruskal Wallis and Dunn's pair comparison test.

RESULTS

Plain control (Group I), showed no pathological sign. In Group II (Negative control) where ulcer was induced by indomethacin (20 mg/kg) once daily for 5 days, the ulcer score was found to be 1.08 ± 0.27 . The ulcer scores in pre-treated standard and test groups where the animals were treated with Standard drug and test drug in low dose were found to be 1.16 ± 0.30 & 1.33 ± 0.27 respectively when compared to negative control which showed non-significant result. In pre-treated test Group B (Group V), the test drug was given orally in the dose of 248 mg/kg, ulcer score was found to be 0.66 ± 0.27 with respect to negative control which showed non-significant decrease in post treated standard group (Group VI). Ulcer score was found to be 0.66 ± 0.27 (insignificant) with respect to negative control. In post treated test group A (VII) it was observed to be 1.08 ± 0.27 (insignificant). In post treated test group B (VIII) score was found to be 0.33 ± 0.10 (insignificant). The ulcer index in negative control pre and post treated standard, test group A and B were found to be 1.25, 1.63, 1.80, 0.44, 0.17, 1.67 and 0.22, respectively, and percentage of ulcer reduction in pre and post treated standard, test group A and B were observed to be -7, -19, 39, 39, 0, and 47, respectively when calculated with negative control (Table 1).

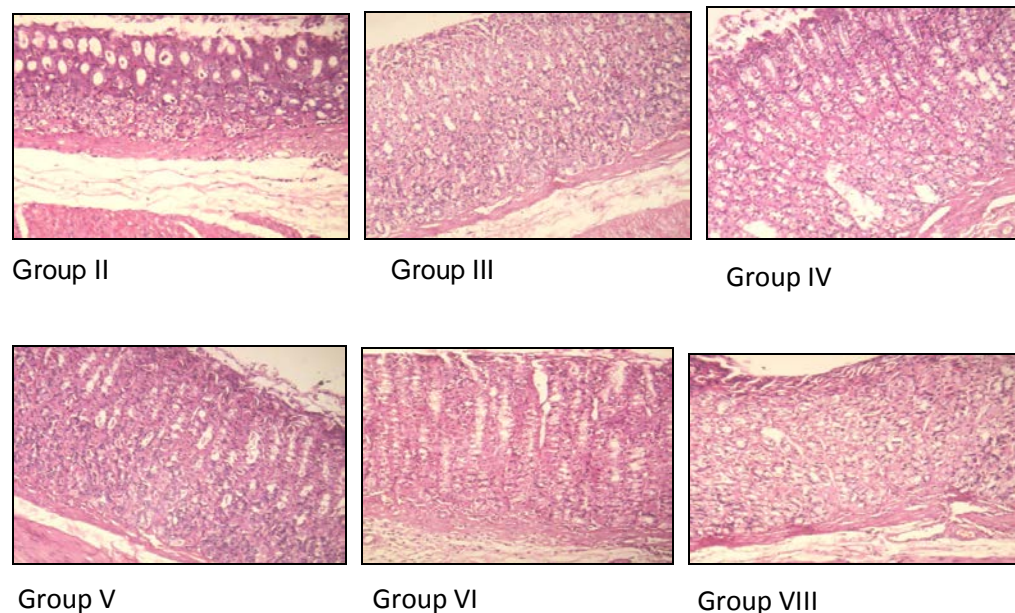


Figure 2. Histopathological slides of different groups (water IMMERSION-induced restraint ulcer model): group II (necrosis, inflammation and ulceration); group III: (necrosis and inflammation); group IV (necrosis and inflammation); Group V (necrosis and inflammation); group VI (necrosis and inflammation); group VII (Inflammatory changes); group VIII (Inflammatory changes).

Table 1. Effect of hydro alcoholic extract of post sumaq on indomethacin induced restraint gastric ulcer.

Groups	Treatment	ADU (Mean± SEM)	(%) RU	Ulcer index	(%) Reduction
Group I: Plain control	DW	0.08±0.08	17	0.01	94
Group II: Negative control	DW + IM 20 mg/kg dissolved in CMC	1.08±0.27	100	1.25	-
Group III: Pre-treated Stand	Omeprazole 20 mg/ kg + IM 20 mg/kg dissolved in CMC	1.16±0.30	100	1.63	-7
Group IV: Pre-treated test A	Post sumaq 145 mg/kg+ IM 20 mg/kg dissolved in CMC	1.33±0.27	100	1.80	-19
Group V: Pre-treated test B	Post sumaq 248 mg/kg IM 20 mg/kg dissolved in CMC	0.66±0.27	67	0.44	39
Group VI: Post-treated stand	IM 20 mg/kg dissolved in CMC+ omeprazole 20 mg/kg	0.66±0.27	50	0.17	39
Group VII: Post-treated test A	IM 20 mg/kg. dissolved in CMC + sumaq145 mg/kg	1.08±0.27	100	1.67	0
Group VIII: Post-treated B	IM 20 mg/kg. dissolved in CMC + post sumaq 248 mg/kg	0.33±0.10	67	0.22	47

(N=6 in each group. DW = Distilled water, IM = Indomethacin, CMC= Carboxy, methyl cellulose, %RU =Percentage of rats with ulceration, ADU = Average degree of ulceration).

Ulcer score in Negative control was found to be significantly increased ($p<0.01$) 1.16 ± 0.21 when compared to plain control. The ulcer score in pre-treated standard, test group A and test group B, score was found to be 0.91 ± 0.27 , 0.66 ± 0.16 , and 0.83 ± 0.21 respectively.

No significant reduction was observed when compared to negative control. While in post- treated Group VI, Group VII and Group VIII first ulcer was graded and ulcer score was found to be 0.41 ± 0.08 , 0.75 ± 0.25 and 0.75 ± 0.25 respectively, but no significant reduction was observed

Table 2. Effects of hydro alcoholic extract of *Post Sumaq* on Water-immersion induced restraint gastric ulcer.

Groups	Treatment	ADU (Mean± SEM)	(%) RU	Ulcer index	(%) Reduction
Group I: Plain control	DW	0.08±0.08	17	0.01	93
Group II: Negative control	DW+ Ulcer induction	1.16±0.21*	100	1.42	-
Group III: Pre-treated Stand	Omeprazole 20 mg/ kg + Ulcer induction	0.91±0.27	83	0.76	45
Group IV: Pre-treated test A	Post sumaq 145 mg/kg+ Ulcer induction	0.66±0.16	100	0.67	60
Group V: Pre-treated test B	Post sumaq 248 mg/kg + Ulcer induction	0.83±0.21	100	0.83	50
Group VI: Post-treated Stand	Ulcer induction + Omeprazole 20 mg/kg	0.41±0.08	83	0.35	75
GroupVII: Post-treated test A	Ulcer induction + Post sumaq 145 mg/kg	0.75±0.25	83	0.76	55
Group VIII: Post-treated B	Ulcer induction. + Post sumaq 248 mg/kg	0.75±0.25	83	0.83	55

(N=6 in each group. Test used Kruskall Wallis test with Dunn'pair comparison test, N= 6* p<0.05 with respect to plain control, D.W= Distilled water).

when compared to negative control. The ulcer index in negative control, pre and post treated standard, test group A and B was observed to be 1.42, 0.76, 0.67, 0.83, 0.35, 0.76, and 0.83 respectively, and percentage of ulcer reduction was found to be 45, 60, 50, 75, 55, and 55 respectively (Table 2).

DISCUSSION

Gastric ulceration has long been viewed as the disease of stress, hence central nervous system may also play role in production of ulcer by causing hyperacidity. The techniques of restraint in albino rats provide a model for the study of stress induced gastric ulceration. Water immersion induced restraint gastric ulcer model is suitable to see anti stress effect of drugs. Therefore, the test drug was also evaluated by using this model. In water immersion induced restraint gastric ulcer model, the test drug was found both precautionary and therapeutic in pre treated and post treated test groups at both dose level but the result was statistically non significant. The histopathological findings are also in consonance. The findings indicate that the test drug does not possess anti anxiety properties and the same has not also been mentioned in Unani classics.

However, it is clear from the result that the test drug has preventive and curative effect only at higher dose. Phyto chemicals in *R. coraria* are ellagic acid, gallic acid is oqueritrin, myricitrin, myricetin, quercetin, quercitrin and tannic acid and flavinoids. Flavonoids protect the

gastrointestinal mucosa from lesions produced by experimental ulcer models and different necrotic agents. Several mechanisms of action may be involved in this protective effect. Quercetin has an anti secretory mechanism of action. However, the most important mechanism of action responsible for the antiulcer activity of flavonoids is the antioxidant properties. Tannins are gastro protective which are present in the drug in sufficient amount (Abu-Shanab et al., 2005; Duke et al., 2003).

As per the Unani theories, it seems that the drug may have acted by temperament, as the Mizaj of the test drug is cold whereas that of diseases is hot (Hubal, 2004; Sina 2007; Tabri, 2000). But the anti ulcer mechanism cannot be understood by Mizaj or phytochemicals. But in the case of herbal drugs, only one or two or more phytochemicals are responsible for action. A number of chemicals and other interventions play the role in exerting actions and the ultimate effect is the cumulative effect. Further study is needed to establish the mechanism of anti ulcer effect of post Sumaq.

CONCLUSION

Results of different experimental models revealed post sumaq to be a promising anti ulcerogenic drug. The test drug possesses curative effect at higher dose against indomethacin induced gastric ulcer. In water immersion-induced restraint gastric ulcer model, the effect was less prominent therefore it can be concluded the test drug

does not possess anti anxiety effect as this model produces ulcer due to stress. This is also evident from the literature that post sumaq is not used as an anti anxiety. The preventive effect of the test drug was more pronounced. This also validates the claim that herbal drugs are more preventive in nature. The drug is more effective at higher dose; therefore, the dose of post sumaq should be revised after toxicity studies.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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Full Length Research Paper

***Wuchereria bancrofti* antigenaemia among school children: A case study of four communities in the Kassena-Nankana east district of the upper east region of Ghana**

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Lymphatic filariasis (LF), a parasitic disease caused by *Wuchereria bancrofti*, is of public health concern especially in the northern part of Ghana. Since 2000, several rounds of mass drug administration (MDA) against this infection have been conducted in the endemic communities. However, no studies on the prevalence of *W. bancrofti* antigenaemia have been conducted among pre-school and school-age children in these communities. This study therefore investigated the prevalence of *W. bancrofti* antigenaemia among pre-school and school-age children in the Kassena-Nankana East (KNE) district between December, 2010 and May, 2012. The study was a cross sectional analytical survey among the school children of age between 2 and 10 years old. Blood samples from two hundred (200) children each (randomly selected from Biu, Korania, Gumongo and Manyoro communities in KNE district) were screened for the presence of *W. bancrofti* antigen before and after MDA was conducted. *W. bancrofti* antigenaemia was detected among 25 (12.5%) children before MDA while 13 (6.5%) children tested positive for the antigen after MDA. The microfilaria antigen prevalence among the communities after MDA were 0% in Biu and Korania, 4.0% in Manyoro and 22% in Gumongo. This study has demonstrated that community variations exist in the prevalence of filarial antigen in KNE district. There is the need for regular surveillance that will inform treatment coverage and effectiveness.

Key words. Lymphatic filariasis, *Wuchereria bancrofti*, antigenaemia.

INTRODUCTION

Lymphatic filariasis (LF) is a parasitic disease caused by nematodes (*Wuchereria bancrofti*, *Brugia malayi* and

Brugia timori). The preferred habitats of these parasites are the lymphatic vessels and lymph nodes where they

induce the development of disfiguring and debilitating clinical symptoms (Rocha et al., 2009). LF is transmitted by a wide range of mosquitoes, depending on the geographic area (CDC, 2010). In Africa, the most common vector is the *Anopheles* species, but *Culex quinquefasciatus* which is the main vector in the Americas is also very common in transmitting LF in urban and peri urban areas in East and Central Africa (CDC, 2010). *Aedes* and *Mansonia* can transmit the infection in the Pacific and in Asia (CDC, 2010). The infection if left untreated, can develop into a chronic disease called elephantiasis and or hydrocele.

The numbers of infected persons are on the increase worldwide due to rural-urban migrations resulting in mushrooming of shanty towns often encouraging formation of favourable mosquito breeding sites (Wamae, 1994). About 120 million people are affected worldwide of whom about 40 million are incapacitated and disfigured by the disease (Wamae, 1994; WHO, 1998). Although, LF is not fatal, it has been ranked one of the world's leading causes of permanent and long-term disability and poverty (WHO, 1998), and can be devastating and crippling at both the individual and community levels (Wamae, 1994). LF is a major public health problem in the Tropics (Harnett et al., 1998; WHO, 2002). LF is endemic over wide geographic areas of Africa, Central and South America, Asia, and Oceania (WHO, 2002; WHO, 1992). *W. bancrofti* is the main species responsible for human LF, and the only known aetiologic agent in Africa (Wamae, 1994). About 751 million people are estimated of being at risk of filariasis infection (WHO, 1992). Of these, about 78 million are already afflicted, and more than 90% of the infected people are believed to harbour *W. bancrofti* (WHO, 1992; Melrose, 2002).

Studies conducted along the coast of Ghana reported 9 to 25% prevalence of *W. bancrofti* microfilaraemia (Dunyo et al., 1996; Gbakima et al., 2005). However, the prevalence of microfilaraemia in many rural communities in the middle and northern parts of the country has been reported to range between 26 to 32% (Gyapong et al., 1994; Dzodzomenyo et al., 1999). The endemic regions include Upper East, Northern, Upper West, Ashanti and Western regions. The highest prevalence rate (36%) was reported in the three Northern regions (Gyapong et al., 1994). In 1998, the World Health Organization (WHO) announced the Global Programme to Eliminate Lymphatic Filariasis (GPELF) with a goal of eliminating LF as a public health problem (Menezes et al., 2007). Lymphatic filariasis is currently subjected to renewed control and elimination programmes (Alexander et al., 2003) using annual mass drug administration (MDA) of

albendazole and ivermectin among all populations that are at risk (Alexander et al., 2003). According to the world health organisation (WHO) technical advisory committee on LF report in 2005, the impact of MDA is variable, ranging from complete interruption of transmission, as in one site in Papua New Guinea where four rounds of MDA had been applied, leading to a significant reduction in transmission as pertains to Ghana and Mali where there have been three rounds of MDA (WHO, 2005).

Monitoring is a vital element in programme management that enables the success of the strategy to be assessed (GPELF, 2000). The MDA programme in Ghana has covered entire endemic population and has completed ≥ 6 rounds in many regions (WHO, 2009) including the KNE district of the Upper East region. The number of districts covered increased from 5 in 2001 to 82 in 2008 (GFELP, 2008). The goals of the Neglected Tropical Diseases Control Programme (NTDCP) of Ghana requires that LF should be reduced to less than 1% among endemic population and antigen prevalence of 0% among children by 2015. The GPELF envisages the elimination of LF globally as a public health problem by 2020. Even though there have been several rounds of MDA which aimed at interrupting transmission thereby reducing the prevalence levels and ultimately eliminating LF, current data on filarial antigen prevalence and the impact of MDA is not available. There is the need therefore, to provide information about the antigen prevalence among school children in some of the endemic communities especially in the Kassena-Nankana East (KNE) district which was one of the few districts to have started the MDA in 2001 (WHO, 2004). This information would be vital for management decision making on the LF elimination programme in the KNE district. The purpose of this study is therefore, to estimate the prevalence of filariasis antigenaemia among pre- and school-age children in four communities in the KNE district of the Upper East Region of Ghana.

MATERIALS AND METHODS

Study site

The KNE District is one of the 12 districts of the Upper East Region. The district which covers an area of 1,675 km² along the Ghana Burkina Faso border is located within longitude 10.51N and 11.02N and latitude 0.92W and 1.55W (Figure 1). It is largely rural and lies in the dry Guinea Savanna wood land with a sub-Saharan climate made up of a wet and a dry season. The wet season extends from April to October, with the heaviest rainfall mainly occurring between June and October while the mean annual rainfall is 1365 mm but

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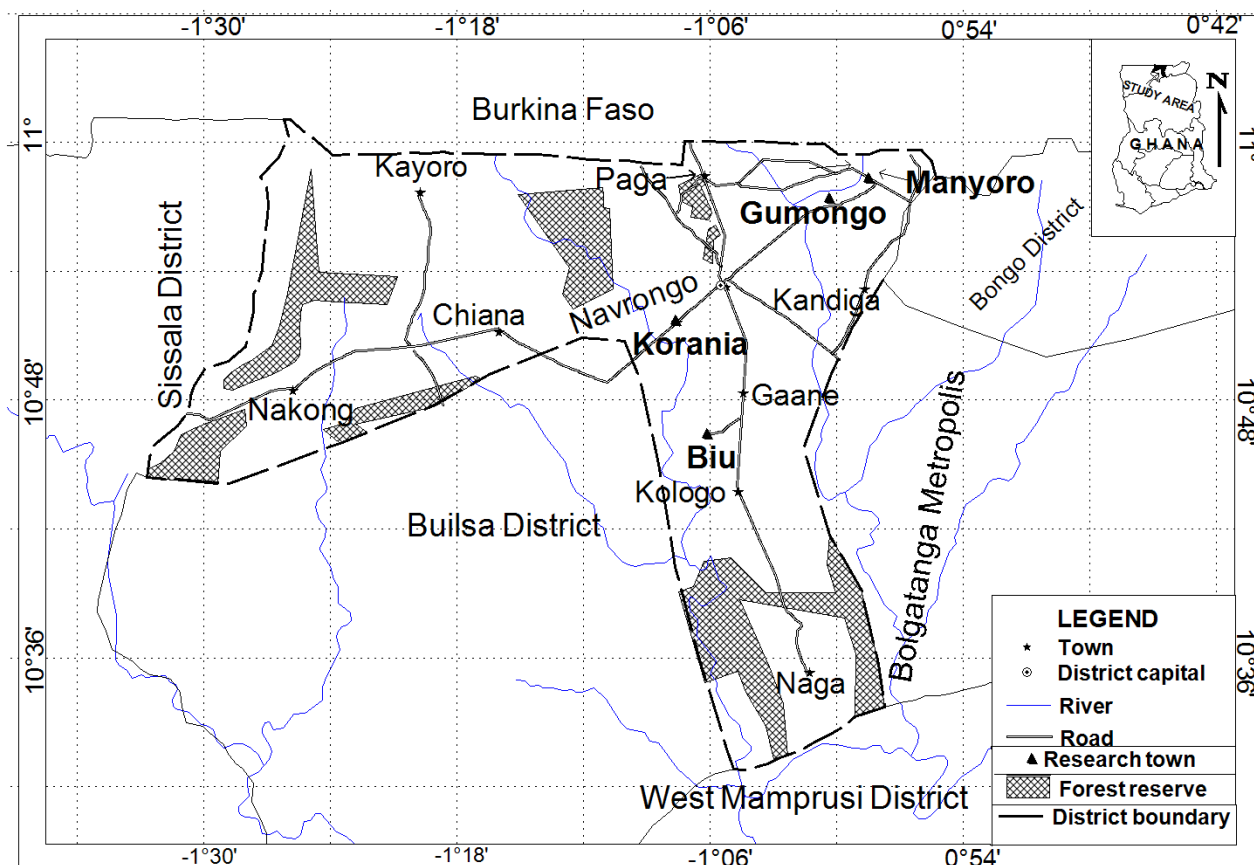


Figure 1. A map showing the study communities in the Kassenan-Nankana East District.

the highest level is recorded in August. Similarly, the dry season is subdivided into the Harmattan (November to mid-February) and the dry hot (mid-February to April) seasons. Monthly temperatures range from 20°C to 40°C (www.kassenanakana.ghanadistricts.gov.gh).

The inhabitants live in dispersed settlements or compounds, protected by outer walls and surrounded by parcels of land for subsistence farming (Gyapong et al., 1996). The district is largely rural, with only 9.5% living in urban quarters (Ngom et al., 1999). The population consists of two distinct ethno-linguistic groups: the Kassena form 49% of the district's population, while the Nankani constitute about 46% with the Builsa and migrants belonging to other ethnic groups making up the remaining 5% (Ngom et al., 1999). The main languages spoken in the area are Kassim and Nankam, with Buili being spoken by most of the minority tribe (Ngom et al., 1999). Despite the linguistic distinction, the population is, in many respects, a homogenous group with a common culture. However the district has ten traditional paramount chiefdoms, and is characterized by traditional forms of village organization, leadership and governance (Ngom et al., 1999).

The study took place in the KNE district which is endemic with *W. bancrofti* infection (Gyapong et al., 1996). In 2001, MDA started at sentinel sites in this district. The prevalence of LF microfilaria in the district before MDA started was 32.4% (Gyapong et al., 1994). However, after more than 5 rounds of MDA, microfilaria prevalence was reduced to 3.5% (Ghana Filariasis Elimination Programme, 2008).

Study population and inclusion criteria

Children born after start of MDA, aged between 2 to 10 years old, residing and attending school in Biu, Korania, Gumongo and Manyoro communities in the KNE district were randomly selected for the study. The Ghana Health Service (GHS) has over the years implemented a number of interventions among the study population; this includes the annual mass deworming exercises with albendazole and ivermectin.

Exclusion criteria

Children who were born before the start of MDA, those not in school and those whose parents/guardians would not give their consent.

Study type and design

The study was a cross-sectional analytical survey conducted in the selected 4 communities in KNE district between December, 2010 and May, 2012.

Study school and participant

Participants were randomly selected from the primary schools of

Biu, Korania, Gumongo and Manyoro communities. These are known LF endemic communities and sentinel sites form as santifilarial drug administration exercises by the Ghana Health Service (GHS).

Sample size determination

A total of eleven primary schools were identified: Biu (4), Korania (2), Gumongo (2) and Manyoro (3). The total children population in the 11 primary schools was approximately 5500 pupil. The initial sample (200 pupils) before MDA as well as the final sample (also 200 pupils) after MDA, were all randomly selected from these eleven primary schools.

Sample collection

A volume of about 2 ml venous blood samples was collected from each participant into a clean labelled heparinised blood collection tubes using conventional venipuncture technique. A vein in the lower arm (cubital vein) was located and the area sterilized by cleaning with cotton wool moistened with 70% alcohol and allowed to dry. A sterile disposable syringe and needle was used to puncture the selected vein and blood was drawn and dispensed into the heparin tube from the syringe without the needle. The collected blood sample were then stored at 2° to 8°C until it was tested for *W. bancrofti* antigen.

Pre/Before MDA sampling

Blood samples of 200 school children aged 2 to 10 years old in the four communities were collected from December, 2010 to January, 2011 before MDA and tested for CFA using NOW ICT filarial antigen kit.

Post/After MDA sampling

Blood samples of 200 school children aged 2 to 10 years in the four communities who have undergone MDA were collected from November, 2011 to January, 2012 and tested for filarial antigen using the NOW ICT filarial test kit.

Laboratory investigations for both pre and post MDA

Information and communications technology (ICT) card test procedure

The NOW® Filariasis version of the card test (ICT filariasis for blood, serum or plasma, patent. no. 5,877, 028; 5,998, 220; 6, 017,767 sensitivity= 100%, specificity =96.37%, efficiency= 96.70%) was carried out according to the manufacturer's instructions. Briefly, each card was removed from the pouch, labelled and laid flat on the work bench. To ensure good blood flow and performance, the capillary tubes were filled with blood. 100 µl of blood sample was slowly added to the pad from the capillary tube. The card was closed after 30 sec to 1 min when the sample has flowed into the pink area and it is completely wet. The result was read exactly 10 min later. The test was considered positive when both lines (test and control) could be read through the visualisation window. Any line (light or dark) appearing in the test position indicates that the result of the test is positive; it is negative when only the control line can be seen.

Limitation of the filarial antigen test

This test is structured to indicate the presence or absence of *W. bancrofti* antigen in the sample. The absence of antigen does not exclude filariasis cause by other nematode species.

Ethical consideration

Signed and informed consent was obtained if the potential child's parent demonstrated understanding of the study after the study has been explained to him/her, and was willing to enrol his/her child. In the case of an illiterate parent, a left thumbprint was obtained on the consent forms and a separate Witness Consent form was signed by a literate witness who had observed the consent processes. The interview was done in Kassim and Nankam, the main local languages in the district. The study protocol was approved by the institutional review board of Navrongo Health Research Centre (IRB NHRC) and Committee on Human Research, Publications and Ethics (CHRPE) of Kwame Nkrumah University of Science and Technology, Kumasi.

Statistical analysis

Data analysis was done using statistical package for social science (SPSS) and MS Excel 2007 software. Data were analysed for the frequencies of filarial antigen test. The prevalence of filarial antigen among the communities, sex and age, multiple Comparisons/Analysis of measurements by community were explored using T-tests and Least Square Difference (SLD); One-Way Analysis of Variance (ANOVA). The predictions of percentages of males and females and age groups testing positive for filarial antigen test were also explored using Binary logistic regression.

RESULTS

Socio-demographic features of the study participants

About 32.5% of the study children were of pre-school level and 67.5% primary school or school-age level. Of these study children, 52.5% were males and 47.5% were females. Majority of these children were of Kassim ethnicity. Prior to the survey, the study children had been treated in the mass drug administration with albendazole and ivermectin by the Ghana Health Service.

Prevalence of filaria antigen before and after MDA

Of the 200 school children selected from the four communities before MDA, 25 tested positive for *W. bancrofti* filarial antigen. This represents 12.5% of the samples analysed (Table 1). After MDA, the overall prevalence of filarial antigen among the study children was 6.5% (13 children). The number of negative filarial antigen recorded in the study was 93.5% (187).

Prevalence of filarial antigen and sex after MDA

Among the 13 children who tested positive for filarial

Table 1. Prevalence of filarial antigen for communities in Kassena-Nankana East District.

Test	Results Before MDA		Results After MDA	
	Frequency	Percentage (%)	Frequency	Percentage (%)
Negative	175	87.5	187	93.5
Positive	25	12.5	13	6.5
Total	200	100.0	200	100.0

Table 2. Prevalence of filarial antigen after MDA and Sex of children.

Gender	Number	Filarial antigenaemia	
		Positive (%)	Negative (%)
Male	105	5 (4.8)	100 (95.2)
Female	95	8 (8.4)	87 (91.6)

Table 3. Prevalence of filarial antigen after MDA and age of the children.

Age group (years)	Number	Filaria Antigenaemia	
		Positive (%)	Negative (%)
1 – 5	65	1 (1.5)	64 (98.5)
6 – 10	135	12 (8.9)	123 (91.1)

antigens after MDA, 8 (8.4%) were females (n=95) and 5 (4.8%) were males (Table 2). However, the difference between the female and the male positives was not statistically significant ($p=0.297$).

Prevalence of filarial antigen and age after MDA

The participating children were categorized into two age groups 1 to 5 and 6 to 10 years. Majority of the participants fall within the 6 to 10 years age group (135 out of 200 children; Table 3). Only one child (1.5%) tested positive for the filarial antigens among the 1 to 5 years age group (n = 65) while twelve from the 6 to 10 years age group were positive. Even though there were more antigen-positive children in the 6 to 10 years age group than the 1 to 5 years age group, the difference was not statistically significant ($p=0.052$). However, binary logistic regression analysis predicts that 1.6% of the children in 1 to 5 years age group will test positive, while 64.2% of 6 to 10 years age group will test positive.

Prevalence of filarial antigen in the communities

Prevalence of filarial antigenaemia in the communities

before MDA was 12.5%. After MDA, the rate reduced to 6.5% (Figure 2). However, among the individual communities, the prevalence after MDA was high in Gumongo (22.0%), followed by Manyoro (4.0%) but none was detected among the participant from Bui and Korania communities (Figure 3). There is significant difference between all the four communities ($p=0.000$). Manyoro and Gumongo are located in the north of the KNE district but are far apart while Bui is down south and Korania in the central part of the district.

DISCUSSION

In spite of the many rounds of MDA in Ghana since 2000, filarial infection continues to affect the people of KNE district with its crippling effect. The success of MDA depends on the fact that children born after the start of the MDA should not be infected of the filarial worm let alone harbour filarial antigens. The purpose of the study was to find out the prevalence of filarial antigen among school children in endemic communities which are sentinel sites for GHS and have undergone several rounds of MDA of single dose albendazole and ivermectin. This is a current report on the prevalence of filarial antigen among school in endemic community in

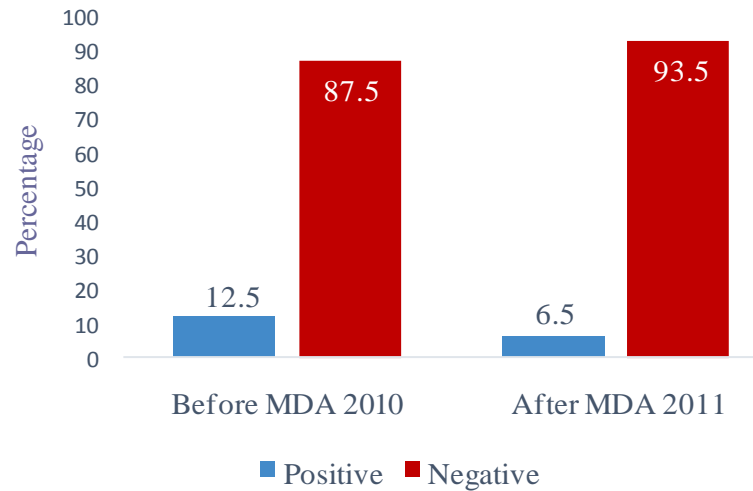


Figure 2. Comparison of prevalence of filarial antigen before and after MDA.

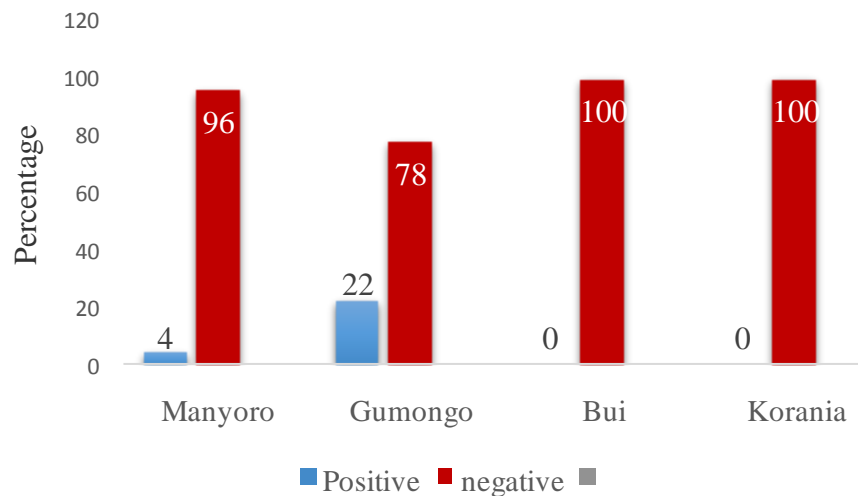


Figure 3. Prevalence of filarial antigen in the communities after MDA.

KNE district.

A study conducted by Gyapong et al. (2002) on the geographical distribution of human infection of *W. bancrofti* in Ghana, Benin, Togo and Burkina Faso revealed over 70% prevalence rates of filarial antigen among adults (age ≥ 15 years) in some communities in Ghana and Burkina Faso. In 2007 however, the Neglected Tropical Disease Control Programme reported 20 to 40% and 10 to 20% LF antigen prevalent rates in northern and southern Ghana, respectively. Our current study, has therefore shown that filarial antigenaemia prevalence in the north has drastically reduced to about 12.5% in 2010 (that is, at the start of our study). After a round of MDA, the rate further decreased to 6.5% among

our studied population.

This reduction in LF antigen prevalence after several rounds of MDA is consistent with the report of Swaminathan et al. (2012) where after eight rounds of MDA, antigen prevalence fell to a range of 0.7 to 0.9% among children aged between 2 to 10 years old. It also compares with another report by Tisch et al. (2008) where after a MDA there was a sharp decline in the presence of *W. bancrofti* microfilaria in individuals who participated in a five year mass drug administration trial in Papua New Guinea. Bui and Korania were among the sentinel sites in the KNE district to have started the MDA and therefore the zero filarial antigen prevalence among children born after the start of MDA in these two

communities is comparable with the observation in Kenya where after several rounds of MDA, (although the annual MDA was not administered in some of the years) the filarial antigenaemia declined from 34.6 to 10.8% with absence of filarial antigen in children born after the start of the programme (Njenga et al., 2011).

The results obtained from this study in Bui and Korania follows a similar result obtained by the Ghana Filariasis Elimination Programme (GFEP) where after an impact assessment carried out for the LF programme demonstrated marked reduction in microfilaria prevalence from 23 to 0.0% (GFEP, 2008). The results of our current study could mean that transmission of *W. bancrofti* infection in Bui and Korania communities have been interrupted, and that these communities have reached the end point in the programme for elimination of lymphatic filariasis. Manyoro and Gumongo had relatively high prevalence 4.0 and 22.0%, respectively. Geographically, these communities are faraway from Bui and Korania, with very bad terrains (that is, bad road network) which sometimes get cut off from the other communities especially in the rainy seasons. This therefore, leads to a situation where not all children in these two communities are able to access the chemotherapeutic agents during the MDA. Moreover, some members of these communities according to Gyapong et al. (1996), do not believe in the scientific cause and interpretation of filariasis, and therefore do not accept the fact that drugs could help treat or prevent them from getting filariasis. They rather perceived and attributed the disease to spiritual causes and hence do not patronize the MDA programmes. In their opinion also, people get hydrocele from ordinary fevers and not through mosquito bites. This also impedes on vector control measures in the communities (Gyapong et al., 1996).

The high level of filarial antigen in children born after the start and repeated rounds of MDA in these two communities is consistent with the finding of Weil et al. (2008) where although infection rates decreased in children after MDA, many young children tested positive for circulating filarial antigen even after three rounds of MDA. This is because MDA did not start in the communities at the same time as with Bui and Korania. Most children in Manyoro and Gumongo started accessing the MDA drug quite late, and hence, many might have long been exposed to the parasite before the start of MDA in their community.

An important finding of the study is that gender does not play any significant role in susceptibility to filarial infection, which is in contrast with a report in Brazil by Medeiros et al. (1999) where men were said to be more susceptible to LF than women. The extent of exposure to the mosquito vectors may rather play a role in the rate of susceptibility to the infection as suggested by the report of Gyapong (2000) where in the southern part of the Ghana, women who were engaged in fishing and trapping

of shrimps in the mangrove swamps were more exposed to the filariasis mosquito which probably resulted in higher prevalence of lymphoedema in women than in men in the region.

CONCLUSION

This study has demonstrated the absence of filarial antigen among children born after the start of MDA in Bui and Korania but not in Manyoro and Gumongo communities. Community variations in prevalence of *W. bancrofti* infection exist in the KNE district. Many other communities in the district must also be monitored to appropriately estimate the prevalence of filarial antigen in the district. Education and other good implementation strategies of controlling LF transmission must be adopted in communities such as Manyoro and Gumongo. Long term follow-up studies must also be conducted to ensure that *W. Bancrofti* transmission is controlled.

LIMITATIONS

The study was limited by not too large sample size due to unwillingness of some parents to release their wards to participate in this study because some parents do not believe in the scientific basis of filariasis.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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