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Case Report Open Access

Normal Pregnancy and Lactation in a Cat after Treatment of Mammary Gland Tumor When Using Photothermal Therapy with Gold Nanorods: A Case Report

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Abstract

Background: Breast cancer therapy, which includes surgery, radiotherapy, chemotherapy and/or systemic therapy can have a profound impact on reproductive functions, leading to loss of fertility. To our knowledge, this is the first report on cancer photothermal therapy using gold nanorods on the mammary gland treatment of a cat, and the safe effect of the treatment on the reproductive function after tumor remission.

Case presentation: A seven years old Shirazi cat (Toatoa) was evaluated because of a 2-week history of progressive dyspnea, signs of depression, and loss of appetite. The cat has a large tumor mass at the left caudal mammary gland. The diameter of the tumor mass was measured using caliper with the dimensions of 14 × 12 × 10.5 cm for length, width and depth, respectively. This was confirmed with ultrasonography. Biopsy samples were taken and fixed in 10% formalin for histopathological investigation, and it was diagnosed as mammary gland adenocarcinoma Grade II. Toatoa was injected intratumoral (IT) with 75 µg gold nanorods (GNRs)/kg body weight followed by exposure to 808 nm laser light for 10 min. GNRs were injected twice, with 15 days apart. After 15 days from the first GNRs injection, there was 60% ablation of the tumor size, while, after 17 days from the second GNRs dose, there was a complete tumor remission. Ultrasound scanning revealed complete ablation of the tumor mass. Complete blood picture (CBC), liver and kidney function analyses showed no changes in any of the tested parameters and indicated that GNRs photothermal treatment is safe and have no immediate toxic effects. After complete remission of the mammary gland tumor, the cat restores all the biological activities including the reproductive function. After 2 months from complete tumor remission, the cat was pregnant after mating with a fertile male, and 62 days later she delivered 3 kittens of normal morphology and growth rate. Breast feeding was found to be normal from all the nipples including the previously affected nipple.

Conclusion: Photothermal therapy with gold nanorods can be used for the treatment of mammary gland tumor with apparently no impairments of reproductive functions in cats.

Keywords: Cat; Mammary gland tumor; Photothermal therapy; Gold nanorods; Pregnancy; Lactation

Introduction

Breast cancer (BC) is the most common malignancy, and its prevalence increases with age in women worldwide. In the developed countries, BC represent more than 40% of all cancers patients of <40 years [1]. In Egypt, BC is estimated to be the most common cancer among females accounting for 32.0% of their total [2]. In addition, dogs and cats have a high prevalence of mammary tumors (MTs), and tumors are more aggressive in cats [3]. Feline MTs (FMTs) comprise approximately 11% of feline neoplasms, are more commonly malignant than benign, highly aggressive, carry a poor prognosis attributable to a high probability of local recurrence and metastasis, and mainly hormone receptor-negative cancer [4-6]. Furthermore, feline and canine mammary tumors have epidemiological, clinical, morphologic and prognostic features similar to those of human breast carcinoma, and it is considered as an excellent model for hormone-independent human breast cancer [7]. Therefore, it is necessary to identify new approaches that can be used for treatment of mammary tumor in pets. According to our knowledge, this is the first report on the application of photothermal therapy with gold nanorods used in feline mammary gland tumor.

Conventional treatments of breast cancer are surgical removal and/or chemotherapy [8,9]. Alternative treatments are hormone therapy and/or directly control function of estrogen receptors [10,11]. The adverse effects of chemical substances and postsurgical metastasis usually result in a shorter survival time.

The application of nanotechnology in cancer therapy research

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started with the use of gold silica core-shell nanoparticles by Halas's group [12] and the use of gold nanorods by El-Sayed group [13]. In both of these studies, the nanoparticles absorb near infra-red light and convert it into thermal energy that ablates the cancer cells. Due to the ease of its synthesis, and the purity of the nanoparticles, the gold nanorods have been used successfully on the small animals (mice and rats) [14]. Intratumoral injection of gold nanoparticles followed by near infrared laser treatment resulted in tumor ablation in treated mice [15-18]. The present study is the first report on the application of photothermal therapy using gold nanorods on large animals. It work reports on the photothermal therapy using the gold nanorods to treat the mammary gland tumor of a cat.

Current therapies, while improving the overall survival rate with mammary tumors, have not eliminated this disease as an important cause of morbidity and mortality. In addition to the high risk of cancer relapse [19], BC therapy could have a profound impact on the reproductive function, and may leads to loss of fertility. In women, BC survivors have the lowest chance for future pregnancy [20]. Only 4-7% of patients with conventional BC therapy actually conceive [21]. The side effects of chemotherapy include damage to ovarian follicles [22], leading to temporary or premature menopause and infertility [23]. Another concerns about pregnancy outcomes after BC therapy, is the presence of inherent risks on the babies born after treatment of BC. To our knowledge, the present case represents only the first case to be reported on the pregnancy and lactation outcomes after remission of feline mammary gland treated using photothermal therapy using gold nanorods.

We here discuss for the first time a report on the treatment of feline mammary gland tumor using photothermal therapy using gold nanorods, and the possibility for the normal pregnancy and lactation after tumor remission, and to evaluate the possible toxic effect of GNRs on blood profile, liver and kidney functions.

Case Presentation

A seven years old Shirazi cat (Toatoa,) sexually intact with previous pregnancy came to a private Vet. Clinic, with 2-week history of progressive dyspnea, signs of depression and loss of appetite, as she was suffering from a large mammary gland tumor at the left caudal nipple.

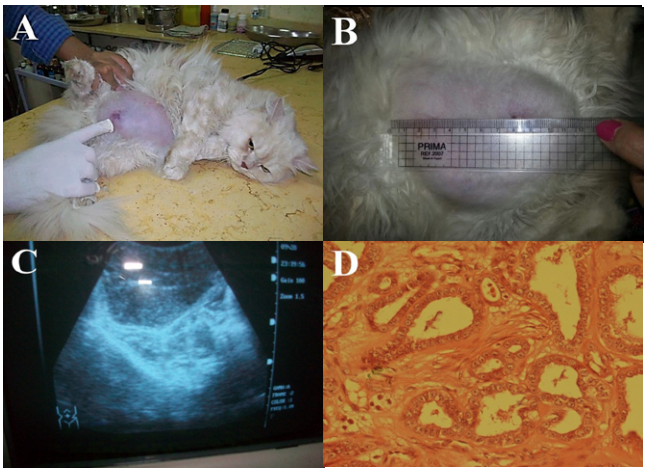

Figure 1: Photographs showing the cat with mammary gland tumor covering the whole abdomen (A), with 12 cm width (B), as confirmed using ultrasonography (C). Histopathological examination revealed glandular type adenocarcinoma Grade II (D).

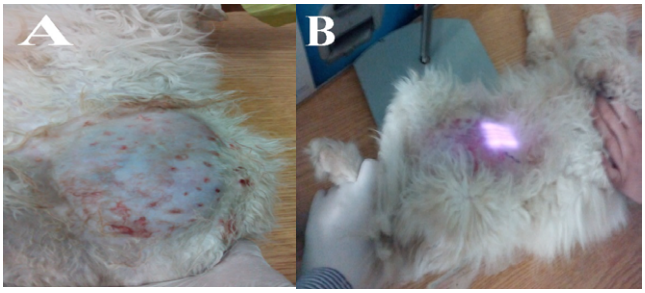

Figure 2: Photograph showing the mammary gland tumor after injection of GNRs (A), and during the exposure to laser (B).

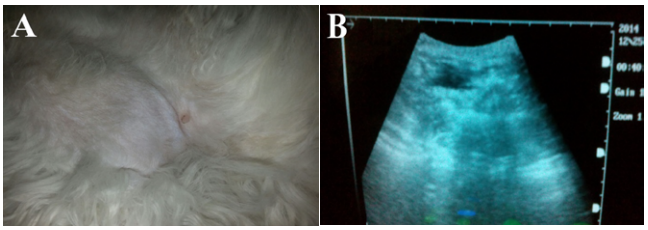

Figure 3: Photograph showing the cat with complete remission of the tumor (A) and was confirmed by ultrasound scanning (B).

The tumor was covering the whole abdomen (14.0 cm length, 12.0 cm width x 10.5 cm depth, Figure 1A and 1B). The cat was examined for the history of mammary gland tumor two months earlier. The cat was checked for physically examined for body weight, temperature, heart and respiratory rates. Also, the tumor size (length, width and depth) was measured by using a caliper followed by ultrasonography examination (Figure 1C). The cat was injected with 0.25 ml Ketamine and 0.25 ml Zylaject for sedation. Biopsy samples were taken from the tumor mass using true-cut needle for histopathological examination. Tissues were fixed in 10% formalin, processed by embedding in paraffin blocks, and then sliced into 5 μm in thickness. Hematoxylin-eosin staining was performed. Images were acquired using Olympus microscope equipped with color CCD camera. According to the case history and histopathological examination the cat was suffering from mammary gland adenocarcinoma Grade II (Figure 1D).

Gold nanorods (GNRs) were prepared at the Spectroscopy Department, NRC, according to the method developed by Nikoobakht and El-Sayed [24]. The dose used in the present study was chosen according to previous literature reports [14]. The cat was subjected for two sessions of treatment. In the first session, 75 μg/kg body weight was diluted in 20 ml physiological saline solution, then injected intratumoral (Figure 2A). Ten minutes after GNRs injection, the site of injection was exposed to continuous laser beam (λ=808 nm) from the top at a power density of 50 mW/cm^2 for 10 min (Figure 2B). After two weeks the same dose and method of injection was repeated. After administration of GNRs, the cat was examined day after day for survival and evidence of behavioral or motor impairments. Also, changes in tumor shape and size were recorded.

After treating Toatoa with 2 sessions of GNRs injection followed by laser exposure (2 weeks apart), a complete remission of the tumor mass was achieved after 31 days from the first injection (Figure 3A). Ultrasound examination revealed complete ablation of the tumor in response to treatment (Figure 3B), and the cat restored her vital activity

including the reproductive one. The cat was mated with a fertile male 70 days after complete recovery, and delivered after 62 days of pregnancy 3 kittens with normal morphological and health conditions (Figure 4A), as well as normal growth rate. Breastfeeding of the kittens was normal including the previously affected teat (Figure 4B).

Blood samples were collected before and one month after treatment for evaluating liver and kidney functions as well as hematological profile. Two blood samples were collected by direct vein puncture, the first was collected in vacuutainer tube containing EDTA for CBC, and the second one was used for serum separation. Whole blood was centrifuged twice at 3000 rpm for 10 min in order to separate serum. Using autoanalyzer (Moduler Analyzer Series, Roche Diagnostics, USA), serum biochemical analysis was carried out to determine the serum level of total bilirubin (TBIL), direct bilirubin, albumin, alkaline phosphatase (ALKP) and serum glutamic-pyruvic transaminase (SGPT) as a measure of hepatic and biliary functions. Nephrotoxicity was assessed by determination of the levels of uric acid (URIC), urea nitrogen (UREA), and creatinine (CREA).

Hematological autoanalyzer (Exigo EOS Vet, SE-126 13 Stockholm, Sweden) was used to determine hematological parameters such as red blood cells (RBC), white blood cells (WBC), hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red blood cell distribution width, neutrophils, lymphocytes, monocytes, eosinophils, basophils, and platelets counts. Analyses of these metabolites in serum of the cat treated with GNRs before and after treatment indicated no difference

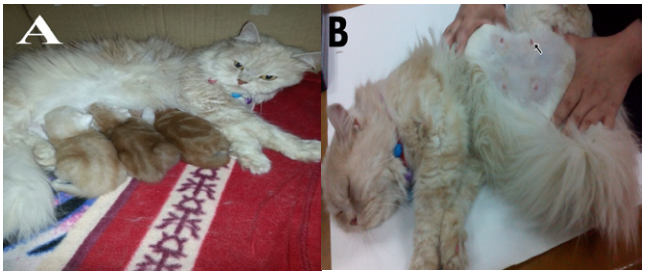

Figure 4: Photograph showing the cat (Toatoa) after parturition and feeding her kittens (A), with the previously affected nipple (arrow) after treatment with GNRs (B).

Items	Normal range	Serum levels		
		Before treatment	1 month	2 months
Liver function tests				
Bilirubin total	0.2-1.2	0.21	0.26	0.24
Bilirubin direct	0.0-0.3	0.03	0.11	0.11
SGPT	Up to 50	37	27	22
Alkaline phosphatase	<390	27	36	34
Albumin	3.8-5.4	3.5	4	3.3
SGOT	Up to 37	32	27	29
Kidney function tests				
Blood urea	18-45	36	37	29
Serum creatinine	<1.5	1.14	1.27	1.25
Serum uric acid	3.5-7.2	0.2	0.2	0.2

Table 1: Liver and kidney function before and after treatment of mammary gland tumor in a cat using photothermal therapy with gold nanorods.

Items	Normal range		Values		
			Before	1 month	2 months
RBCs					
RBC	5	11	8.72	10.46	7.54
HCT	25	45	39.8	37.5	29.4
HGB	8	15	12.5	14.9	10.6
MCV	39	50	45.6	35.9L	39
MCH	12.5	17.5	14.4	14.3	14
MCHC	31	38.5	31.5	39.8H	36
RDW%	14	18.5	20.5H	22.0H	21.9H
RDWa	20	43	30.5	23.7	25.9
WBCs					
WBC	5.5	19.5	5.2L	10.4	10.1
LYM%	0	99.9	23.4	19.3	9.9
MON%	0	99.9	11.2	11.4	5.5
NEU%	0	99.9	65.6	68.6	84.6
EOS%	0.1	99.9	0	0.7	0
LYM=	0.9	7	1.2	2	1
MON=	0.3	1	0.6	1.2H	0.5
NEUT=	3.5	13	3.4	7.2	8.6
EOS=	0.1	99.9	0	0.0 L	0
PLT=	200	500	219	239	231
MPV=	8	12	8.1	8.6	8.5

Table 2: Complete blood picture before and after treatment of mammary gland tumor in a cat using photothermal therapy with gold nanorods

was observed in any of the tested parameters. Liver and kidney functions were the same before and after treatment (Table 1). Also, values of blood profile were the same before and after treatment (Table 2).

Discussion

There is some controversy about the number of women that became a pregnant after BC diagnosis. Personal fears or lack of appropriate and rapid fertility counselling probably contribute to this observation. Positive association between abortion and breast cancer was frequently reported from case-control studies [25,26].

After 2 weeks of IT injection of 75 µg GNRS/kg body weight, there was 60% reduction in tumor size. A second dose of IT injection of GNRs was given, and there was a complete remission of the tumor after 31 days from the start of treatment. Similar results were previously reported in rats and mice [14]. Previous results showed that the AuNRs can generate defects in the cell membrane and induce apoptosis of cancer cells [27]. Moreover, thermal therapy with NIR laser and gold nanoparticles has proven effective in breast cancer cell lines *in vitro* [28,29] and in mice [14].

Interestingly, the cat restored her biological activities after tumor remission, and it became pregnant after mating with a fertile male (60 days after complete remission). She delivered three kittens in a good health and normal morphology. The best available retrospective evidence suggested that pregnancy after BC does not increase the risk of disease recurrence [30-32]. Recently, among risk factors for breast cancer, a history of full-term pregnancy was inversely associated with the risk of second primary cancer [33]. Also, breastfeeding was normal, particularly for the previously affected nipple. Similarly in women, there is no evidence that breastfeeding increases the risk of breast cancer recurrence or a second breast cancer development [34,35]. According to our knowledge this is the first report discussing the effect of pregnancy after mammary gland tumor remission in pet animals.

The present results revealed that IT injection of GNRs has no toxic effects on blood, liver or kidney functions in the treated cat. RBCs, WBSc count or constituents, also, total and direct bilirubin, SGPT, albumin and alkaline phosphatase, none of these parameters showed any statistical difference before (control) and after treatment. This result indicates that the use of GNRs is non hepatotoxic, nephrotoxic and does not affect blood profile. Although no comprehensive studies evaluating the toxicity of gold nanorods have yet been reported, all available evidence indicates that at physiological doses gold nanorods are not cytotoxic and pose no short–term health risks. Previous results indicate that these GNPs did not promote toxic effects on hepatic tissue [36-38]. Also, much higher doses of gold nanoparticles (2700 mg/kg) appeared non-toxic to experimental animals as well [39]. Yet, *in-vivo* toxicological studies indicated no mortalities, significant weight changes nor adverse effects in mice treated with PEGylated GNRs [40]. In contrast, naked GNPs are harmful to liver both in *in vivo* and *in vitro* conditions [41]. Other results have suggested that toxicity of GNPs appears to be related to the choice of the coating materials and/or to their size [42,43]. Pan et al. [42] recorded that 1.4 nm GNPs capped with triphenylphosphine monosulfonate were much more cytotoxic than 15-nm GNPs capped with the same material. The same author reported that the toxicity of small GNPs depends on their ability to trigger the intracellular formation of ROS [43].

Conclusion

Photothermal therapy using gold nanorods could be a novel, non-invasive and safe approach for treatment of mammary gland tumors in cats without impairment of subsequent pregnancy and lactation.

Declaration of Interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research presented.

Acknowledgment

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Author Contributions

Abdoon AS design the work; and Abdoon AS, Al Ashkar EA, Kandil OM, EL Ashkar MR, Shaban AH conducted the experimental work; Eisa WH prepared GNRs; EL Shaer MA conducted the histopathological examination; and Shaalan AH, Hussein KH and El Sayed M. revised the results and experimental work; Abdoon wrote the paper. Every author read and annotated the paper.

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