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Evaluating the Effects of Gastric By-Pass Surgery and Sleeve Gastrectomy, as New GDV Treatment Modalities, on Vitamin B12 Values in Dogs

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ABSTRACT

The purpose of this study is to evaluate the effects of gastric bypass surgery and sleeve gastrectomy on vitamin B12 values in dogs. Gastric dilation and volvulus (GDV) is an acute disease with a high mortality rate in dogs. Either gastric bypass surgery or sleeve gastrectomy can be utilized as surgical treatments for GDV. These techniques can provide increased success and fewer complications, however, one undesirable outcome can be vitamin B12 insufficiency. Three groups of dogs received gastric surgeries, one group underwent gastric rotation and derotation with no tissue gastric incisions, the two other groups had gastric bypass surgery and sleeve gastrectomy. Vitamin B12 level was evaluated two weeks prior to surgery, at the time of surgery, two, four, and six weeks after surgery. The results demonstrated no inter-group differences two weeks prior to surgery until four weeks after surgery. However, six weeks post-operation, significant differences between the sleeve gastrectomy group, and the other two groups were shown in vitamin B12 levels. Gastric bypass surgery and sleeve gastrectomy can both be utilized as new surgical managements of GDV. However, as we have demonstrated in this article, an important expected side effect is vitamin B12 insufficiency, in dogs that receive sleeve gastrectomy for GDV treatment. This necessary element must be substituted artificially for all dogs which had received these surgical managements.

Introduction

Being overweight is an abnormal and excess of fat accumulation in different parts of the body.[1] According to statistics in year 2008, 1.46 billion people around the world are categorized as overweight, and 502 million are considered obese.[2] With increased prevalence of overweight people, there has been an increase in the number of related medical conditions, as diabetes, stroke, osteoarthritis, cardiovascular diseases and cancers.[1] Nowadays bariatric surgery is widely performed on human subjects by two major techniques, which has been done with the least side effects:

During gastric bypass surgery (GBS) a new communication is created between small intestine and stomach. This surgery is most efficient for losing weight in human patients. Following surgery, with the reduction of absorbing surface of the digestive tract and

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food intake, weight loss occurs. Through gastric by-pass surgery, stomach and duodenum is bypassed from digestive tract, and by reducing the gastric volume and absorbing surface, weight loss occurs.[3] The second method is sleeve gastrectomy (SG). During this surgery a major segment of stomach including the fundus is excised and removed. This part of stomach includes secretory cells which produce appetite hormones. Through this surgery, following the reduction in stomach volume and appetite hormones, weight loss occurs.[4]

In human studies, it has been shown that surgeries on gastric tract, including bariatric surgeries and removing the stomach, either totally or partially, result in reduction in vitamin B12 absorption. Vitamin B12 is a water soluble vitamin which can be found either in food or supplements.[1,2,5,6] Normal value of vitamin B12 in dogs is 251-908 ng/liter.[7] Vitamin B12 is necessary for red blood cell production, neural function and DNA synthesis.[1,3,5,6] Vitamin B12 in the food is released by gastric hydrochloric acid and protease in stomach. When vitamin B12 is added artificially to the food or is used as supplements, it is in its free form and doesn't need the releasing stage. The next step is conjugation with gastric intrinsic factor. Intrinsic factor is a glycoprotein secreted by parietal cells of stomach. The resulting combination is finally absorbed in the distal segment of ileum. The sufficiency of vitamin B12 is measured by evaluating the amount of serum or plasma vitamin B12. In human cases the serum values less than 120-180 picomol/liter is considered insufficient.[3]

Surgical procedures on digestive tract, including bariatric surgeries and techniques during which, gastric tissue is removed either partially or totally, result in losing hydrochloric acid and intrinsic factor releasing cells.[3,8,9] These surgeries reduce the absorption of vitamin B12, especially the vitamin B12 in food. Vitamin B12 insufficiency is the most common ingredient insufficiency in patients who had received gastric by-pass surgery.[4] Potential complications resulting from vitamin B12 insufficiency include anemia, fatigue and general weakness, neural and cerebral dysfunctions.

In veterinary medicine, Gastric dilation and volvulus (GDV) is an acute disease with a high mortality rate in dog breeds. Following this condition, the stomach may undergo partial or total necrosis and total or segmental gastrectomy would be inevitable.[10] GVD needs urgent intervention as it accompanies a noticeable mortality rate, as high as 10%. Breed, age, chest form, diet, etc. are among the risk factors related to GVD.[11] In treating GVD, the purpose is focused on returning cardiovascular and subsequently the renal function. All interventions are done to reduce the tension of stomach on abdominal vessels and lungs.[12] In management of patients with GVD, lines of treatment respectively include less invasive interventions as in fluid therapy, antibiotic therapy and cardiac anti-arrhythmia medical therapy and somehow more invasive but temporary treatments to reduce gastric pressure as orogastric tube insertion, percutaneous needle decompression and eventually temporary paracostalgastrotomy.[13] In surgical management of GDV, if the gastric tissue is undamaged, surgery is performed to return the stomach to its original position and fixation to re-establish digestive tract.[14] If gastric tissue had undergone necrosis, surgery would include excision of necrotic tissues. In that case, either GBS or SG can be utilized. These novel techniques with reduced surgery time and increased sufficiency can correlate with increased success and less undesirable complications.

Considering the undesirable effects of vitamin B12 insufficiency in human patients who had received either GBS or SG, in this study we have evaluated the effects of these two modern gastric surgeries on vitamin B12 values in dogs.

Materials and Methods

Animals

For this study, 15 mixed breed dogs were included, with age ranging from 2 to 5 years and weighing from 18 to 24 Kg. 6 dogs were male and the remaining 9 dogs were female. Inclusion criteria were general health, complete growth, and receiving anti-parasite and vaccination 2 weeks prior to surgery, and normal results in thyroid hormones and blood sugar tests. Anti-parasite drugs included Caniverm tablet (each tablet contained 150 mg Fenbendazolum, Pyranteli embonas 144 mg, Praziquantelum 50 mg/10kg liveweight, PO, q2wk, Bioveta, Czech Republic) and Ivermectin (200 µg/kg, SC, single dose, Alfasan, the Netherlands). Exclusion criterion was any previous surgery. The whole study was conducted due to animal experiment rules assigned by Shiraz University ethics committee (issued in 24.12.2017 number 9360430).

Surgery

For the study the dogs were divided into three equal groups randomly. Each dog was controlled with a leash,

and received preanesthetic sedation, acepromazine (0.05 mg/kg, IM, single dose, Alfasan, the Netherlands). The dogs were situated on surgical table in dorsal recumbent position. General anesthesia was induced by ketamine (5 mg/kg, IV, single dose, Bremer Pharma, Germany) and midazolam (0.2 mg/kg, IV, single dose, Caspian Tamin, Rasht, Iran), combined in a single syringe and maintenance was done by partial re-breathing technique with halothane with minimum alveolar concentration (MAC) of 0.9% combined with oxygen (Noble Pharma, Ontario, Canada). Cefazolin (22 mg/kg, IV, q1h, Loghman, Tehran, Iran) was started 30 minutes prior to surgical incision.

In purpose of preparation for surgery, the hair on chest and abdominal area were clipped and then shaved completely. Surgical area was disinfected and prepared with povidone-iodine 10% and draped with sterile drapes. In each case, an incision was made with a number 10 surgical blade from sub-xyphoid to pubis in both skin and linea alba to access abdominal organs, and a second incision parallel to inferior rim of 13th ribs in all layers of skin, fascia and muscle to further increase the access to intra-abdominal organs, with enough access to stomach.

In the first group of dogs after laparotomy and exposing abdominal tissues, the stomach was rotated and de-rotated. An undesirable and artificial clockwise 270-degree rotation was induced manually, held for 1 hour, and then was de-rotated manually into primary position. In the second group after laparotomy and intra-abdominal surgical access, gastric bypass surgery was performed. Through surgical access to stomach, cardia was identified. A circumferential full thickness incision was made in stomach, with 50 ml volume containing gastric tissue remaining attached to cardia, detaching the rest of stomach from cardia. In order to keep the gastric content apart from surgery site, two clamps were placed on either side of the incision. The proximal incision was sutured in two layers with Vicryl 2-0 with Cushing and Lembert techniques to invert the incised edges of the wound, from either ends of the tissue held with clamp, the middle third of the incised tissue was not sutured and simply was maintained with the holding clamp until the further steps of the surgery. The distal side of the incision, the whole incision line was sutured in two layers with Vicryl 2-0 with Cushing and Lembert techniques to invert the incision edges. Forty centimeters distal to pylorus, and distal to duodenum, two clams were placed on intestine to keep digestive content inside the tract, and a full thickness circumferential incision was done through intestine. The distal edge, held with the clamp was sutured to the middle unsutured edge of the incision proximal to cardia, in one layer with Vicryl 2-0 with simple interrupted sutures, using end-to-end technique.

After that, a 3 centimeters long full thickness through and through incision was made 100 centimeters proximal to the junction of the small intestine and colon as a new opening in digestive tract. The proximal edge of the incision in duodenum, which was still held with a clamp, was sutured to this new opening with Vicryl 2-0 in one layer with simple interrupted sutures using side-to-end technique. In order to make sure that all of the suture sites were water proof, in cardia region, the leakage test was performed by placing a feeding tube in the esophagus, extending to the cardia region, and 50 ml of normal saline containing methylene blue was administered under pressure. None of the cardia sites showed any signs of leakage. Also in ileum, the leakage test was performed by placing two Doyen clamps on either side of the suture site 2 centimeters away from the incision and injecting 10 ml normal saline solution containing methylene blue by a 24-gauge needle.

For the third group of dogs SG was performed as explained in the following. The stomach was exposed from cardia to pylorus. To keep the digestive content inside the stomach, a doyen forceps was placed on the greater curvature of the stomach from cardia to pylorus, in a form that the remaining gastric passage in the digestive tract, has the same internal measures as the duodenum. A full thickness incision was made lateral to the forceps and the lateral segment of the stomach was excised and removed. The incision line was sutured in two layers with Vicryl 2-0 using Lembert and Cushing techniques to invert the edges of the wound. The forceps were removed and to confirm that the incisions were sutured water tightly, a feeding tube was placed inside the esophagus and 50 ml of methylene blue containing saline was administered. No signs of leakage appeared on sutured sites. After surgical interventions on digestive tract, the abdominal wall incision was sutured in 3 layers of fascia, muscle and sub-cutaneous with Vicryl 2-0 by simple continuous stitches; and skin was closed in one layer with nylon 0 by simple interrupted sutures. Sterile dressing with petrolatum gauze was applied on the surgical site.

Skin sutures were removed after 15 days under sedation with acepromazine (0.05 mg/kg, IM, single

dose, Alfasan, the Netherlands). The dressings were changed daily since the first day post-operation. To keep the animals from licking the surgical site, and avoid contamination with saliva, Elizabeth collar was applied on animals for 15 days. Post-operation, cefazolin (22 mg/kg, IM, q12h, Loghman, Tehran, Iran) was administered for 7 days. During the first five days of post-operation admission and monitoring time, per each day, the dogs received intravascular 1/3-2/3 serum 10 ml/hr/kg for the first 12 hours and ringer lactate serum 10 ml/hr/kg for the second 12 hours. From the sixth post-operation day until the eleventh day, the dogs received high protein, high calorie and no fat diet every 12 hours from Island yogurt 400 gr with 36 gr protein and 0% fat (Kalleh Diaries, Tehran, Iran). 200 grams of yogurt was diluted with 200 ml water and fed to the dogs.[15] Following the eleventh day until the sixth week, the animals were fed with 250 gram of dry dog food with high protein (29%) and low-fat content (Nutripet, Tehran, Iran) twice a day. Drinking water was available for dogs since the first day after surgery.

Laboratory Evaluation

Serum samples were obtained from each animal two weeks before the surgery, at the time of the surgery, two weeks, four weeks and six weeks after the surgery to evaluate B12 level. Serum samples were obtained in 5 ml volume before meal under antiseptic circumstances. The samples were transferred to a human hormonal laboratory to determine vitamin B12 level using enzyme-linked immunosorbent assay (ELISA) method by DRG International diagnostic kits (DRG Diagnostics, Marburg, Germany).

Statistical Analysis

The measured results were inserted in an SPSS file version 17 for statistical data analysis. Comparison of vitamin B12 levels between different groups at similar times was assessed using ANOVA ($p < 0.05$ was considered statistically significant).

Results

The mean value of vitamin B12 level in each group of animals is demonstrated for every time interval in Table 1. Table 2 demonstrates inter-group differences in serum vitamin B12 levels in similar times. Comparing the three methods of surgery on vitamin B12 values in different times, the measured serum parameters demonstrated no inter-group significant differences at two weeks prior to surgery, at the time of surgery, two weeks and four weeks after surgery. Yet, the results showed significant differences in vitamin B12 levels between group C and the two other groups, six weeks after surgery. The vitamin B12 in serum samples of dogs in group C was statistically less than the two other groups after six weeks. ($p < 0.05$).

Table 1. Mean value of vitamin B12 level (ng/liter) in animal groups in different times.

	Two weeks prior to surgery	At the time of surgery	Two weeks after surgery	Four weeks after surgery	Six weeks after surgery
Group A	278.4	336.0	317.6	308.2	328.8
Group B	297.8	329.0	324.8	291.6	288.0
Group C	303.4	360.4	284.2	260.4	221.6
Total	293.2	341.8	308.9	286.7	279.5

$p < 0.05$ is considered statistically significant.

Discussion

In veterinary medicine GDV is an acute and potentially lethal disease with considerable mortality rate. Following this unpleasant condition, the stomach may go under necrosis and subsequently partial or total gastrectomy can be inevitable.[10] During surgical treatment of this disease, in cases with healthy gastric tissue, stomach derotation and fixation can be the ultimate treatment.[14] In those with gastric necrosis, during surgery, the necrotic tissue must be excised and removed. Either GBS or SG can be utilized. These two novel techniques can increase success and decrease undesirable complications through reduced surgery time and elevated surgical effectiveness.

In this study we have evaluated short term effects of different surgical treatments of GDV on serum vitamin B12 level. Since we have enrolled stray dogs in this study, some of these animal models already suffered from vitamin B12 deficiency at the beginning of the study (mean serum vitamin B12 level was 293.2 ng/liter with four dogs showing vitamin deficiency in laboratory data). Therefore, all animal samples were fed with high protein and complete vitamin content diet from two weeks prior to surgery until six weeks after surgery. Serum vitamin B12 level reached normal range at the time of surgery in all of the animals, with the mean value reaching 341.8 ng/liter and no serum sample showed vitamin B12 deficiency (the serum

Table 2. Evaluation of inter-group difference in serum vitamin B12 level (ng/liter) in each time category.

Time	Sample group (I)	Sample group (J)	Mean difference (I-J)	Std. error	Sig.	95% confidence interval for mean	
						Lower bound	Upper bound
Two weeks prior to surgery	Group A	Group B	-19.4	39.75307	0.878	-125.4558	86.6558
		Group C	-25	39.75307	0.807	-131.0558	81.0558
	Group B	Group A	19.4	39.75307	0.878	-86.6558	125.4558
		Group C	-5.6	39.75307	0.989	-111.6558	100.4558
	Group C	Group A	25	39.75307	0.807	-81.0558	131.0558
		Group B	5.6	39.75307	0.989	-100.4558	111.6558
At the time of surgery	Group A	Group B	7	36.5929	0.980	-90.6249	104.6249
		Group C	-24.4	36.5929	0.787	-122.0249	73.2249
	Group B	Group A	-7	36.5929	0.980	-104.6249	90.6249
		Group C	-31.4	36.5929	0.676	-129.0249	66.2249
	Group C	Group A	24.4	36.5929	0.787	-73.2249	122.0249
		Group B	31.4	36.5929	0.676	-66.2249	129.0249
Two weeks after surgery	Group A	Group B	-11.4	31.69543	0.932	-95.9591	73.1591
		Group C	33.4	31.69543	0.559	-51.1591	117.9591
	Group B	Group A	11.4	31.69543	0.932	-73.1591	95.9591
		Group C	44.8	31.69543	0.365	-39.7591	129.3591
	Group C	Group A	-33.4	31.69543	0.559	-117.9591	51.1591
		Group B	-44.8	31.69543	0.365	-129.3591	39.7591
Four weeks after surgery	Group A	Group B	16.6	18.87609	0.663	-33.7588	66.9588
		Group C	47.8	18.87609	0.063	-2.5588	98.1588
	Group B	Group A	-16.6	18.87609	0.663	-66.9588	33.7588
		Group C	31.2	18.87609	0.262	-19.1588	81.5588
	Group C	Group A	-47.8	18.87609	0.063	-98.1588	2.5588
		Group B	-31.2	18.87609	0.262	-81.5588	19.1588
Six weeks after surgery	Group A	Group B	40.8	16.58312	0.072	-3.4415	85.0415
		Group C	107.2*	16.58312	0.000	62.9585	151.4415
	Group B	Group A	-40.8	16.58312	0.072	-85.0415	3.4415
		Group C	66.4*	16.58312	0.005	22.1585	110.6415
	Group C	Group A	-107.2*	16.58312	0.000	-151.4415	-62.9585
		Group B	-66.4*	16.58312	0.005	-110.641586	-22.1585

* indicates significant difference with other groups.

samples were taken just before the operation). Also, high protein and sufficient vitamin level were maintained in post-surgery diet. Since all the dogs showed increase in serum vitamin B12 level in two weeks period before surgery and no deficiency was identified at the time of surgery, it was concluded that none of the animals had any problem in producing and releasing intrinsic factor in digestive tract.

Serum vitamin B12 level was also evaluated every other week post-operatively until the sixth week. Serum vitamin B12 level in dogs which had received SG were statistically less than other two groups on the sixth week post-operatively. But the measurements didn't demonstrate any significant difference in less than six weeks from surgery. Moreover, among the five dogs included in this group which underwent SG, after four weeks from surgery, three dogs showed vitamin B12 insufficiency; and after six weeks from surgery, four dogs showed vitamin B12 insufficiency in paraclinical evaluations.

Natural vitamin B12 in food is released by gastric protease and hydrochloric acid. When vitamin B12 is added to food artificially, as complement, is in free form and does not require this releasing step. After releasing, vitamin B12 binds with gastric intrinsic factor, which is a glycoprotein secreted primarily by pancreas and secondarily by parietal cells in stomach. The final combination is absorbed through endocytosis in distant segment of ileum. Considering the fact that during SG surgery, a noticeable volume of stomach is excised and removed, the decrease in mucosal intrinsic factor production and subsequent decrease in serum vitamin B12 level can be expected. In none of the surgeries applied in this study, pancreatic tissue or its secretary apparatus into digestive tract was manipulated, and we have only focused on the effects of decreased mucosal

intrinsic factor, whether it can be significant or negligible. The dogs were fed with nutrition rich with vitamin B12 during this study, therefore we have evaluated the digestive tract sufficiency and ability in absorbing this vitamin.

In this study we have considered the dogs which only received rotation and derotation during surgery, as the control group, since no intervention was performed on gastric tissue, therefore no change in vitamin B12 digestion or absorption was expected. Nevertheless, in surgical treatment of dogs with GDV, if irreversible damage has happened to the stomach, the need to excise and remove the necrotic tissue would be inevitable.

The two novel surgical techniques of SG and GBS can both be utilized in treating dogs with GDV. Upon the anatomic site of damage, it may be possible for the surgeon to choose either techniques or be obligated to use one. Sleeve gastrectomy, in compare to gastric by-pass surgery, is simpler to execute and less time consuming, therefore there is less chance for intra-operative complications to occur. Accordingly, sleeve gastrectomy can be most surgeons' first choice in surgical treatment of dogs with GDV. The surgeons must consider vitamin B12 issue post-operatively, by modifying nutrition and supplementary diet.

As demonstrated in this study, vitamin B12 absorption from food in animals which had received SG reduces noticeably post-operatively. The consequences of vitamin B12 insufficiency includes: anemia, fatigue, general weakness, neural and brain dysfunction. Since both SG and GBS are common bariatric surgeries performed on human patients, several studies have evaluated their effects in human medical fields, yet no controlled study have been done in animal models considering vitamin B12 serum levels following theses surgical techniques.

Similar studies on human patients have shown similar results with this study. In 2006, Crookes conducted a study which depicted cobalamin reduction post-operatively in bariatric surgeries. Again in 2006, Ballantyne demonstrated reduction in protein absorption following obesity surgery.[3,8,9]

Since substituted artificial vitamin B12 still requires gastric intrinsic factor for absorption, the physician must notice providing this factor in patients who has received gastric surgeries, in which physiological intrinsic factor production, release or combination with digestive material may have been altered.

GBS and SG can both be utilized as new surgical managements of GDV. These two techniques have been utilized in human bariatric surgeries for many years with little side effects and noticeable advantages. Following SG and GBS, an important expected side effect is vitamin B12 insufficiency. Vitamin B12 is crucial for red blood cells production, neural function and DNA synthesis.

This necessary element must be substituted artificially for all dogs which had received these surgical managements to eliminate GDV. Furthermore, gastric intrinsic factor must also be added to their diet since it may not be efficiently produced or released over digested food as the gastric tissue goes under surgical tissue resection.

Acknowledgement

None.

Conflict of Interest

None.

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