Serum globulin compensation index can predict favorable prognosis in canine mammary malignancy

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Abstract

A new index for globulin compensation (GCI) was developed and tested for its prognostic value by investigating the serum protein alterations in bitches with mammary gland tumors. Serum proteins were estimated in twenty bitches with mammary gland tumors before surgery and the results were compared with the data from a group of twenty clinically healthy bitches served as control by using student's t-test. Significantly ($p<0.05$) lowered level of serum total proteins and albumin were observed in bitches with malignant mammary tumors with elevated ($p<0.05$) total globulins compared to controls. A mathematical formula i.e GCI was developed and tested for the first time in canine mammary tumors by analyzing the survival data of bitches obtained from the clinical laboratory records followed by classification of cases into three categories: negative GCI and negative compensation (low GCI), GCI of 0 to <1 with partial compensation and GCI = or >1 with full compensation (elevated GCI). Cases with elevated GCI (in the range of 0.6-1.12) were observed to have favorable prognosis than cases with low GCI (<0.5) indicating the reliability of globulin compensation index for predicting the prognosis in canine mammary malignancy.

Keywords: globulin compensation index; canine; mammary malignancy; prognosis.

Introduction

Mammary gland tumors are the most frequently diagnosed tumors in bitches and constitute about half of all tumors in bitches (Rivera *et al.* 2009). The origin of canine mammary tumor is complex and the development of the tumor may be influenced by both genetic and environmental factors (Egenvall *et al.* 2005) but the exact mechanisms that lead to the origin and development of mammary tumors are unknown (Rivera *et al.,* 2009). Alterations in plasma protein level are observed in various pathological conditions like malignancy, inflammation, hepatic disorders and chemical injury (Ceron *et al.* 2005). Albumin is the most abundant protein in plasma constituting nearly 60% of the total protein content. Albumin is synthesized in the liver and performs vital functions in the body like binding and transport of substances to target tissue, osmotic regulation and buffering activity. In tumor bearing animals, albumin synthesis is decreased due to reduced transcription of albumin gene or changes in albumin mRNA processing. Measurement of serum albumin level can be used for screening, progression and response to treatment of different cancerous conditions including canine mammary tumors (Tecles *et al.* 2005). Globulins are heterogeneous group of proteins with distinct functions and are classified into alpha, beta and gamma globulins based on their electrophoretic mobility. Globulins are synthesized in the liver and lymphoid tissue. Alpha and beta globulins are synthesized in liver where as plasma cells and lymphocytes in lymphoid tissue produce gamma globulins. Globulins contribute to the binding and transport of different substances in the body and immune response. Increase in globulins level is observed in cancerous conditions to compensate the albumin loss. Previous reports also indicated the importance of serum globulins in diagnosis and studying the progression breast cancer and canine mammary tumors (Al-Jaudi 2005, Ahmad and Ali 2007 and Tecles *et al.* 2009). In addition to the cancer conditions, changes in the concentration of albumin, globulins and albumin: globulin ratio has been observed in various disorders in animals making it non-specific for diagnosis and prognosis of tumor conditions. In malignant conditions it is observed that, extra hepatic tissues synthesize globulins in an amount not enough to compensate the severe hypoalbuminaemia in these conditions. Thus, measuring the compensatory capacity of serum globulins will be more sensitive and specific in predicting the clinical outcomes in malignancy conditions than estimating only serum albumins, globulins and albumin: globulin ratio. Several other clinical markers have been also identified as diagnostic and prognostic markers for canine mammary tumors including enzymatic and hormone specific markers, specialized proteins, gene specific markers, immunohistochemistry based markers etc. However, these markers have some limitations in veterinary clinical practice, particularly in remote field conditions due to their high cost of testing, lack of adequate infrastructure and specialized scientific manpower for their estimation or testing, instability and due to unavailability of testing materials also.

Hence, this study was planned to develop a cost effective clinical index based on the compensatory capacity of globulins in canine mammary malignancy, which can be tested and used in the field conditions without having any sophisticated laboratory facility. A mathematical formula was developed for the first time in bitches with mammary malignancy to express the extent of globulin compensation in case of reduced serum albumin and was also tested for its prognostic value in canine mammary malignancy.

Material and methods

Ethical approval
This study has been approved (112/VBC/IAEC) by the Institutional Animal Ethical Committee (IAEC).
Animals
Twenty nine bearing malignant mammary gland tumors depending on their histopathology, ultrasounds, X-rays were selected from the Veterinary clinical complex, College of Veterinary Science, Rampura Phul, Bathinda. Clinical histories of the bitches were obtained from clinical reports. For control group, twenty nine clinically healthy adult bitches were selected from the Veterinary clinical complex, College of Veterinary Science, Rampura Phul, Bathinda coming for routine checkup.
Sampling
Collection of blood samples
Two mililitres of blood were collected from cephalic vein in sterilized centrifuge tube from adult healthy female dogs (n=29) and those bearing mammary tumors (n=29). Serum was used for estimation of different proteins.

Collection of tumor tissue samples

Fresh mammary tumor tissues (n=29) were collected in tissue collection vials from the bitches immediately after surgery and were rinsed with ice cold normal saline solution to remove any red blood cells and clot. Then the mammary tumor tissues were fixed in 10% neutral buffered formalin (NBF) and sent to the Laboratory for histopathological analysis.

Estimation of serum protein profile

The level of total proteins and albumin in serum were estimated using Autopak kits on semi-automatic biochemistry analyzer. The concentration of total globulins was obtained by subtracting the concentration of albumin from the concentration of total serum proteins.

Collection of data for establishing the serum globulin compensation index (GCI)

The data used in this study for developing the GCI were collected from the records of the Veterinary clinical complex, College of Veterinary Science, Rampura Phul, Bathinda. The clinical records of twenty nine bitches analyzed for protein profile above and died of mammary gland tumor (Deceased group) were observed and investigated statistically. All these bitches had low serum albumin levels measured within four month period preceding their death. The control group was included with twenty bitches suffered from mammary malignancies (Survivor group) and was followed up nearly four months after measurement of their serum albumin level. Bitches having history of hemorrhage, inflammation and infection, those received blood and blood components before estimating serum albumin levels were not included in the study.

Establishment of the GCI

GCI was calculated and developed from the ratio of deviation of serum globulins (G) from normal, to the deviation of serum albumin (SA) from normal. The following mathematical formula was obtained:

$$GCI = (G - 2.3)/ (3.1 - SA)$$

(G and SA: the measured serum globulins and serum albumin respectively, and factor 2.3 and 3.1 were the minimum of their normal ranges obtained in g/dL respectively).

Depending on their serum GCI scores canine patients were categorized into:

Category I: negative GCI and negative compensation (G <2.3 g/dL)

Category II: GCI of 0 to <1 (G more than or equal to 2.3) and total serum protein <6.0 g/dL with partial compensation and

Category III: GCI = or >1 (G > 2.3) and total serum protein more than or equal to 6.0 g/dL with full compensation.

Statistical analysis

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) software. Significant differences between different groups with respect to various parameters were determined by the Student's t-test. The p-value less than 0.05 ($p<0.05$) was considered as significant.

Results and discussion

The measured level of different serum proteins (Table 1) in bitches with mammary tumors indicated that total serum proteins and albumin obtained for malignant group was significantly ($p<0.05$) lowered compared to corresponding control group however, the level of globulins was observed to be significantly ($p<0.05$) increased in malignant tumor bearing bitches. GCI for each case within each category was also calculated for the deceased and survivor groups. The percentage of death was observed to be high i.e 52.6% with only 16.5% survivors in category I having low GCI value. In category III where there was full globulin compensation the percentage of death was recorded to be only 15.7% with 51.4% survivors where as in partial compensation category percentage of survivors and deceased were almost equal.

The statistics of serum albumin (table 2) indicated significant difference ($p<0.05$) between the two groups with a p-value of 0.031. The survivor and deceased group also differ significantly ($p<0.05$) with respect to their GCI values (table 3). The ratio of mean serum albumin of survivors to that of deceased was 1.35 where as the ratio of mean GCI of survivor to that of deceased was calculated to be 2.96. Comparing the mean values it clearly indicates that globulin compensation index is 2.19 times (2.96/1.35) more sensitive than serum albumin.

Table 1. Serum protein changes in bitches with malignant mammary gland tumors. Data are presented as mean ± SE of 29 independent experiments performed in duplicate.

Parameters	Control group (n=29)	Malignant group (n=29)
Total protein (g/dL)	6.25±0.03	4.81±0.40[*]
Albumin (g/dL)	3.12±0.03	0.49±0.02[*]
Globulins (g/dL)	2.21±0.04	3.83±0.04[*]

Control group: Clinically healthy bitches;   n= Number of bitches
[*]Indicates a significant difference (p<0.05) compared to corresponding healthy controls

Table 2. Statistical distribution of serum albumin (g/dL) in survivors and deceased groups.

Groups	No. of animals	Mean ± SD	Significance
Survivors	16	2.35 ± 0.25	0.031
Deceased	13	1.74 ± 0.39	

Survivors group (Control); Significance p<0.05; SD: Standard deviation

Table 3. Statistical distribution of globulin compensation index (GCI) in survivors and deceased groups.

Groups	No. of animals	Mean ± SD	Significance
Survivors	16	0.86 ± 0.26	
Deceased	13	0.29 ± 0.15	0.023

Survivors group (Control); Significance p<0.05; SD: Standard deviation

Serum contains a number of proteins performing variety of functions in the body. Decrease in total serum proteins observed in present study may be due to poor nutrition and mental distress associated with malignancy and poor quality life during disease condition (Barber et al. 2000). Decrease in total serum proteins has been observed in many breast cancer patients compared to healthy women (Al-Mudhaffar and Al Samarai 2001, Ahmad and Ali 2007). The decline in total serum proteins observed in this study in tumor bearing bitches was in line with the previous studies. Decreased albumin concentration is frequent in serum of patients with cancer however the exact reasons are not understood (Tecles et al. 2005). Some studies had reported decreased serum albumin in women with breast cancer and animals with metastasis (Ahmad and Ali 2007 and Tecles et al. 2009). Reduced serum albumin level can be used as an indicator of prognosis in several diseases, including cancer, kidney diseases and chronic liver diseases (Al-Jaudi and Wahab 2004, Al-Jaudi 2005). The low level of serum albumin observed in the present study in tumor bearing bitches can be the main reason of decreased total serum proteins in these patients, as albumin constitutes a major fraction of total serum proteins. Serum globulins estimation is also helpful in diagnosis of specific diseases including breast cancer. Increase in the level of globulins reported in our study was in agreement with reports of the previous workers which might be due to excess formation of various globulins by cells to provide protection against the increased tumor load in bitches (Al-Mudhaffar and Al Samarai 2001).

Protein synthesis is an essential cellular process and the types and quantity of proteins produced differs according to body demands and availability of factors required for synthesizing proteins. Alterations in the protein synthesis capacity have been observed in different pathological conditions which also affect the protein content of serum. The disease where protein synthesis capacity of the liver is affected, serum albumin level was reduced which is mainly compensated by the globulins, creating a balance called as albumin: globulin ratio; but due to lack of pathophysiological value its utilization in disease diagnosis has been limited in recent days. The globulin compensation index measures the compensation capacity of serum globulins when albumin level is decreased in bitches with mammary malignancy. In debilitating diseases protein synthesis capacity of the liver is less affected and can be restored by feeding but in critically ill patients where hypoalbuminaemia is common as observed in case of cancers, infusion of albumin does not improve the condition due to systemic illness. Thus, by determining the compensation capacity of globulins in these conditions the prognosis can be easily made. As observed in our study, following decreased serum albumin the serum globulin was increased but not to an extent sufficient enough to compensate the critical reduction in serum albumin in malignancy. In these conditions the index was also successfully tested by comparing the value of globulin compensation with the percentage of survival among the bitches classified under above three categories making this globulin compensation index suitable for predicting the

outcome in canine patients with malignancy of mammary gland. This index can be used as marker in determining the prognosis in bitches with mammary malignancy.

Conclusion

The results of this study indicated that there is prominent alteration in the serum proteins in bitches affected with malignancy of mammary gland. The GCI developed in range of "0.6-1.12" can be useful in predicting the favorable prognosis in canine mammary tumor patients. This investigation opened up the path for performing more studies in this aspect which will be helpful in unraveling the mechanisms behind these alterations and prognostic usefulness of the GCI in mammary cancers as well as other types of cancers with low serum albumin.

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Conflict of Interest

No Conflict of interest.

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