CHAPTER V
DISCUSSION

Chondroitin sulfate is a major glycosaminoglycan (GAG), which mostly found in body such as articular cartilage, bone, skin, cornea and wall of blood vessel. In various connective tissue of the articular cartilage, chondrocyte has function to synthesize, organize and regulate the chondroitin sulfate which is containing in most GAG in extracellular matrix. At normal status of articular cartilage, the relative rates of matrix synthesis and degradation are adjusted to achieve dynamic equilibrium. When joint injury occurs, the cartilage metabolism is imbalanced by enhancing degradation as well as decreasing synthesis that can cause the articular cartilage degradation. Chondroitin sulfate is degraded and released into synovial fluid, and then into blood circulation then it is metabolized in the liver and excreted by kidney. Thus, determination of chondroitin sulfate in serum and synovial fluid reflect metabolism and pathologic process of articular cartilage and help for early diagnosis of osteoarthritis. Immunoassay, by using monoclonal antibody, specific to chondroitin sulfate chain, has been shown the ability that may be the mean of choice in early diagnosis of OA (8, 58-59). Both monoclonal antibodies which recognize unsaturated terminal chondroitin-6-sulfate after chondroitinase ABC digestion such as 3B3 (42, 60-61) and native epitope in chondroitin sulfate chain as WF6 (42, 60) have been studied in order to apply them as biological markers reflecting cartilage metabolism.

Monoclonal antibody WF6 has been produced and developed from Bone and Joint Research Laboratory, Faculty of Medicine, Chiang Mai University which specific to chondroitin sulfate specific patterns that generally found in articular cartilage. Previous studies had investigated the serum WF6 epitope concentration in association with cartilage degradation. In study of human, the result found that serum concentration of WF6 epitope in osteoarthritic patients was significantly higher than normal patients (38, 62). In a study of dogs that underwent experimental transection of the anterior cruciate ligament for induction of OA, an increase in the WF6 epitope concentration was also found in OA dogs (63). In view of horses, it was found that OA horses had the higher

WF6 epitope than in normal (42). In contrast to this present research (study A), OA horses had significantly lower WF6 epitope concentration than normal horses. The lower WF6 epitope concentration of OA horses in our study suggested that average of time which horses were affected by OA were 1 year approximately and most horses in OA group (70.83%) were in an advanced stage of disease which characterized by loss of cartilage mass due to the chronic cartilage metabolism. These may cause the decrease matrix turnover of articular cartilage. Therefore, less chondroitin sulfate would be available for degradation and elaboration into body fluids. This is similar to keratan sulfate determination, as catabolic marker in horses with various joint diseases, the result showed that serum keratan sulfate concentration in OA horses were significantly less than control groups (14).

For comparison of serum WF6 epitope concentration between normal horses and horses with arthritis or OC in this research, as yet there is no study concerning the use of WF6 epitope as biological marker for chondroitin sulfate measurement on both horse groups in Thailand. In the present study, arthritic horses and horses with OC had significant lower WF6 epitope concentration than normal horses, contrary to one study; which reported that plasma keratan sulfate concentration in arthritic and OC horses were significant higher than in control horses (14). From our result, it is possible that some of horses in normal group were not necessarily a true normal for comparison. Although, physical and lameness examinations revealed that they had normal conformation, showed no abnormal clinical sign and lameness, and blood examination showed normal blood chemistry and hematologic value, some of abnormality could be conceal. For example, non-progressive idiopathic cartilage erosion that there is no visible clinical sign, but the articular cartilage erosions can observed as incidental finding on arthroscopic and necropsy examinations (64). In addition, radiographic examination still has had some limitation in early detection of OA. Unfortunately, when OA are recognized by radiography, the structural alterations in the articular cartilage are already irreversible. In arthritic horses of this study, joint injury in research time could not be at first. Perhaps, those horses might have joint trauma several times, frequently found in working horse.

Consequently, inflammation of joint which occurred repeatedly which may contribute to the degenerative process in joint by the release of enzymes, inflammatory mediators and cytokines. This may result in the decrease level of WF6 epitope from normal level. It is similar to horses with OC, the chip fragment was usually still attached to synovial membrane and their movement within the joint caused direct tugging on the synovial membrane. Severe inflammation of the joint had occurred and then articular cartilage was much damaged until it remained in small quantities. Moreover, this present study also found that the radiographic finding of some horses with OC (25%) had the chronic pathologic lesion, and the average of time from occurrence of bone fracture in joint to fragment finding by radiographic diagnosis were 1 month approximately. During which the articular cartilage passed through catabolism for a month period. This may result in the WF6 epitope level of horses with OC were less than in normal horses. Thus, the period of bone fracture and grading of severity in osteochondral fracture are necessary for further analysis. From all results in comparison of serum WF6 epitope concentration between normal horses and horses with arthritis, OC or OA, these indicated that serum WF6 epitope concentration was changed when joint abnormalities had occurred.

Besides, this research found that working normal horses had significantly higher WF6 epitope concentration in serum than no training normal horses. This indicated that working such as racing, show jumping, general riding had effect on the changes of WF6 epitope concentration may due to increased matrix turnover. In this research, the comparison of WF6 epitope concentration in serum among normal horses in various age and breed groups were found, there was no significant difference of the concentration of WF6 epitope in any age and breed groups similarly the previous studies, there was no different significance in this epitope in newborn to more than 15 years olds (42), and age and breed did not have significant effects on plasma KS concentration in clinically normal horses (14). These indicated that age and breed has no influences on the serum WF6 epitope concentration. In previous study of dogs, the comparison of serum WF6 epitope concentration among normal dogs in different groups of body weight and sex, they found that there was no different significance in serum concentration of WF6

epitope between dogs weight less than 10 and more than 25 kilograms. There was no significant difference of the WF6 epitope concentration in serum between male and female dogs (65). These suggested that WF6 epitope may be a reliable marker if we can determine whether it is an anabolic or catabolic marker.

For multivariable analysis, the result showed that there was significant correlation between diseased status of joint or working status and serum WF6 epitope concentration. These indicated that diseased status of joint or working status had influences on the serum WF6 epitope concentration. According to the results, diseased status of joint was negatively correlated, and working status was positively correlated to the WF6 epitope concentration in serum that meant if the horse had joint abnormality, WF6 epitope were in low level and if it had normal joint, WF6 epitope were in high level and if the horse was worked, WF6 epitope were in high level and if it was not worked, WF6 epitope were in low level.

From these results, first; total protein determination that found there was no significant difference of serum TP concentration in all horse groups, second; concentration of serum WF6 epitope/TP in horses with arthritis, OC or OA were significantly lower when compared with normal horses which similar to result in WF6 determination in horses with arthritis, OC or OA, and third; there were significant positive correlations between WF6 epitope and WF6 epitope/TP concentrations in serum. These indicated that WF6 epitope can be represented CS concentration in serum (ng/ml).

In study B, this is a preliminary study by using mAb WF6 for therapeutic monitoring in horse with OC prior to and after arthroscopic surgery. It was found that the OC horse had WBC count more than normal value before treatment which indicated to joint inflammation. After treatment on week 4, WBC count had decreased, but it was still in the level of inflammation. WBC count had gradually decreased to the normal WBC value on week 8, showed the inflammatory process had depleted and joint return to normal status. On week 12, WBC count was increased again, and higher than the normal value and value at pretreatment. This suggested that OC joint returned to inflammation again which conformed to additional history taking and physical

examination. The OC horse had trained by trot for 4 days before the period of rest was complete at week 12. Clinical sign including joint swelling and heat were also found. From WBC result, it was found that WBC count tend to decrease continuously reflected joint inflammation, which arose from pre to post-treatment, was decreased until it was over. If there was no extrinsic factor as the use of work interfering. In view of WF6 determination, there was gradual increase of WF6 epitope after arthroscopic surgery from week 4, 8 to 12. Although, the removal of chip fragment had been done, the inflammatory and catabolic processes of articular cartilage progressed continuously. Though WBC count had gradually decreased to normal value on week 8 indicated that inflammation of joint had stopped; however, the cartilage degradation stimulated by inflammatory process continued. Therefore, this can caused the serum WF6 epitope to be in high level. On week 12, there was more increasing of WF6 epitope apparently conformed with the increased WBC count, when the horse underwent training that led to joint inflammation once again. These results indicated that if horses are used for working prior to appropriate time, the cartilage metabolism will progress to permanent osteoarthritis. All results of this study indicated that when joint was aroused by abnormal mechanical load, infection, aging or osteochondrosis which result in pathologic change of the joint, which can be detected by WF6 epitope. Therefore, WF6 epitope may be suitable for monitoring joint diseases.

Furthermore, the comparison of WF6 epitope/TP concentration in synovial fluid between abnormal and contralateral normal carpal joints in horses with OC showed that the first horse (OC1) had concentration of WF6 epitope/TP in OC joint lower than in contralateral normal joint, contrary to OC2 that had inverse result. In OA horse, it was found that the OA joint had WF6 epitope/TP concentration higher than contralateral normal joint. This suggested, the radiographic examination had reported that the OA joint was in an early stage which the articular cartilage was not much more damaged and it remained in large quantities, therefore WF6 epitope/TP concentration could be found in a high level. Nevertheless, this present study had some uncertain history of horses and sample limitation; including those 3 horses were affected from joint diseases

in unequal time. Therefore, result should be interpreted cautiously. However, the result of this study manifests the changes of WF6 epitope level in synovial fluid when joint has abnormality such as osteochondral fracture and osteoarthritis, it can be used as basic information for further study that investigate this epitope in a sufficiency of sample numbers, including the period of time from bone fracture to diagnostic finding, and grading of OA should be done.

Although, serum collection is simple and lesser invasive when compared with synovial fluid collection. The WF6 epitope in serum is a total quantity of chondroitin sulfate derived from whole body, contrary to in synovial fluid that is the level of chondroitin sulfate which is measured from articualr cartilage directly. Therefore, determination of WF6 epitope in synovial fluid is more specific and reliable than in serum. From one study, it was found that chondroitin sulfate epitope 846 and carboxy propeptides of type II collagen (CPII) concentrations in serum were not significantly correlated with in synovial fluid (8).

From one previous study, Peansukmanee et al found the OA horses had the higher serum WF6 epitope concentration than normal (42) contrary to our study that the OA horses had significantly lower WF6 epitope concentration in serum than normal horses. Interestingly, the level of serum WF6 epitope concentration in normal horses in the previous study and our study were similar, indicated that the assay used in this research was reliable. However, we can not determine whether WF6 epitope is catabolic marker as same as the previous study because the lower WF6 epitope concentration in OA horses than normal and diseased status of joint was negatively correlated to the WF6 epitope concentration in serum. The conclusion that WF6 epitope is anabolic maker is not consistent with the result of our study (study B). Moreover, in view of 3B3 epitope which is anabolic marker, Peansukmanee et al found that newborn horses up to age less than 2 years old had significantly higher 3B3 epitope concentrations when compared with other age groups, which does not agree with our study that there was no significant difference of the concentration of WF6 epitope between any age groups.

At present, there are no any studies which clearly report that the WF6 epitope is a catabolic marker of articualr cartilage. However, the results from previous studies presumed that the WF6 epitope may be an indicator for articular cartilage degradation. In our study, even though this research can not define that WF6 epitope is anabolic or catabolic marker, can conclude that serum WF6 epitope changes according to several factors such as worked load, joint trauma, and joint disease. Therefore, WF6 epitope may be used as biological marker accompanied with other routine diagnostic techniques to indicate articular cartilage degradation and useful for screening test, early diagnosis or monitoring of treatment in equine joint disease.

However, in this research, the causes of a small sample number were from the limitation of research time and difficulty of sample collection. For further investigation, the changes of WF6 epitope accompany with various biological markers such as 3B3, 846 epitope, keratan sulfate in serum and synovial fluid should be studied in a larger sample. Grading of disease severity, types of work and the therapeutic monitoring in a long period of time should be done for more understanding of pathological process or disease activity, to diagnose and plan the appropriate treatment of joint diseases in horse.