CHABTER IV

Discussion

The Study of The ELISA Technique

The major improvement of modified Anderson - Kelly's technique as shown in Figure 10 was the condition of incubating temperature, changed from room temperature to 37°C (water bath). The incubating temperature at 37°C (water bath) showed more suitable curve and less variation than at room temperature. The direct contact of heat carrier of water with ELISA plate reduced the incubation period of the reaction of antiferritin with ferritin and ferritin with peroxidase conjugated antiferritin from 30 minutes as Anderson and Kelly's method to 15 minutes for both reactions (Figures 11-13).

The optimal dilution of antiferritin for coating ELISA plate was 1:1500 and 50 µl of peroxidase conjugated antiferritin in 12 ml of conjugate diluent solution (1:240) was the optimal volume for testing when considered the efficiency and economy of the results as shown in Figures 14 and 15. The dilution of 1:1500 of antiferritin for coating was more economic than the Anderson and Kelly's method which

used the dilution of 1:1000 while the volume of peroxidase conjugated antiferritin was equal to the Anderson and Kelly's method.

The modified Anderson and Kelly's technique was checked for the precision by doing within assay and between assay controls as shown in Figures 16 to 18 and Table 4. The data showed that the variation of high level of serum control was slightly too high to be accepted while the low and medium level of serum control were accepted by the value of % CV which should be smaller than 10. However, the high level of samples could be further diluted for examinations.

The accuracy of the modified ELISA technique was not tested by checking % recovery. However, it was compared with the Radio Immunoassay (RIA) of Gamma dab kit. The coefficient correlation of both methods was 0.987. It showed that in accuracy, the modified ELISA technique was highly agreeable to the RIA technique using Gamma dab kit.

The modified ELISA method has many advantages over the original Anderson and Kelly's method. The incubation period and variation were reduced, because of the direct contact of heat carrier of water to the plate and constant temperature was controled at 37°C in water bath.

The reported ELISA method has several advantages over conventional RIA methods and previously reported ELISA methods. Large numbers of specimens can be processed in a limited space using the microtiter plates which also eliminate the need to label individual tubes. If plates were coated overnight the assay could be completed in less than 2 hours in the case of modified Anderson and Kelly's ELISA method. It was also less hazardous than the RIA technique. Moreover, the lebelling enzyme in the ELISA technique has longer life than the radioactive of lebelling Iodine in the RIA technique. And finally, the method is most economical currently (1986). The cost of an assay done in duplicate is not more than 5 baths per specimen when all the solution in each lot were used.

The RID and The CIEP Technique

The sensitivity of Radial Immunodiffusion (RID) was 10,000 ng/ml, and Counter Immunoelectrophoresis (CIEP) was 3,000 ng/ml. The sensitivity of both techniques were too low to detect the ferritin in the tested serum, while most of samples could be measured by the ELISA technique, the serum ferritin levels were less than 1,000 ng/ml. However, both methods may be appropriate for other diseases such as haemochromatosis and leukemia which have very high serum ferritin level.

In the case of raising concentration of ferritin in serum samples by using lyphogel absorbtion. The technique is too complicate for routine test. Besides these, if the serum were over concentrated, it will be too sticky tc be accurately pipetted and it may cause uneven diffusion from agarose wells.

The Possibility of Ferritin to be Tumour Marker

According to the incidence of lung disease patients is higher among male than female (1,6,48). The samples which were obtained for this study were 65 males and 5 females in all lung cancer, 28 males and 8 females in non-treated tuberculosis and 21 males and 2 females in inflammatory lung diesase. In this discussion, therefore, most of the result being study were from male only.

The mean and standard deviation of serum ferritin level in normal samples were 73.2 and 74 ng/ml in male, 52 and 48 ng/ml in female, respectively. These results were similar to other investigators', both in Thailand and other countries. When the high serum ferritin was considered as mean + 2 S.D. or above (221 ng/ml), the high serum ferritin level was found in all samples from male groups (Table 5).

1. The percentage of male sample showing high serum ferritin level in non-treated, non-metastatic lung cancer was 64.5% and in other lung diseases, divided into inflammatory lung disease, non-treated tuberculosis and pneumonitis were 52.3, 46.4 and 42.8% respectively. It implies that the lung cancer can not be differrentiated from other lung diseases by the levels of serum ferritin, although the unpaired t-test shows significant difference between non-treated, non-metastatic lung cancer and non-treated tuberculosis ($p<0.05$). However, each of the serum ferritin level can not indicate the types of lung disease because the high overlapping rate of serum ferritin level in each lung disease as shown in Figure 25.

From the previous study , Groupp et al, who used anti-placenta ferritin for study, only 14 (37.9 %) out of 37 non-metastasis lung cancer have high serum ferritin. The result of using anti-placenta ferritin can not be well compared with this study (using anti-liver ferritin) because Groupp et al, had not studied the samples of other lung diseases. The anti-placenta ferritin may be more useful than anti-liver ferritin if it have a specificity with only lung cancer. This is an interesting point which required more study in detail. Besides these, using the combinations of various antiferritins should also be emphasized.

2. The usefulness of ferritin level in follow-up of lung cancer after treatment was not studied because of the long period and small success of lung cancer treatment. However, the study for comparison between non-treated, non-metastatic lung cancer and during-treated,non-metastatic lung cancer whose clinical symptoms were better was done. Both unpaired t-test (table 7) and chi-square test (table 8) demonstrated that the ferritin level and numbers of samples showing high serum ferritin level in both sample groups were non-significantly different. The useful of serum ferritin level for follow-up should not be concluded until the study of lung cancer after treatment was done.

3. However, the significant decrease of serum ferritin level and numbers of samples showing high level of serum ferritin between non-treated tuberculosis and tuberculosis after treatment were calculated by both unpaired t-test and chi-square test, respectively (table 7 and 9). This is useful for the follow-up of the treatment of suspected tuberculosis patients (by only chest x-rays) who have been treated for chemotherapeutic diagnosis and also in tuberculosis patients, serum ferritin should be decreased if the treatment was successful.

This may be called non-specific follow-up which is more practical than microbiologic examination and can be used as a confirmed test with x-rays.

4. The patients who were diagnosed to be metastatic lung cancer were compared with non-treated, non-metastatic lung cancer. The serum ferritin level was significantly different between both groups when calculated with unpaired t-test. However, the numbers of this sample group being tested were too low to be accurately interpreated by chi-square test. It is doultful whether the serum ferritin will be useful for diagnosis of the presence of metastatic lung cancer. This point still need further study.

5. The cell types of non-treated, non-metastatic lung cancer were tabulated in table 10, including the serum ferritin level and the percentage of samples showing high serum ferritin level in each cell type. Non-significant difference among each cell type was shown in both level of serum ferritin and number of sample showing high level of serum ferritin when calculated by statistic methods. However, serum ferritin level may be more related to squamous and large cell types more than others. The low numbers of specimens may be the reason for non-significant difference result. It may be elucidated by larger amount of samples.

6. In hepatocellular carcinoma, serum ferritin level was raised in 80.0% of 25 samples of males. This suggests that serum ferritin level which was examined by anti-liver ferritin may be useful for diagnosis hepatocellular carcinoma. However, Melia et al (36) found that 88% of 23 samples of

cirrhosis, serum ferritin level was raised. He also reported that 97% of 35 samples of hepatocellular carcinoma, serum ferritin was also elevated. In his work, it was non-significantly different between the groups of hepatocellular carcinoma and cirrhosis. But, Kew et al (35), have also reported the negative correlation between alpha-faetoprotein (AFP) and serum ferritin level in hepatocellular carcinoma. That is, serum ferritin may be raised in AFP-negative patient of hepatocellular carcinoma and will be useful for follow-up for the responsiveness of treatment.

7. In various types of cancers such as cancers of gastro-intestinal tract, esophagus, breast, cervix and others which were also randomly studied. The results showed that the high level of serum ferritin was found in only 5 cases (4 males and 1 female) of various cancers (n=28). The results were not appreciated and were similar to other investigators' who had also designed their experiment to study the level of serum ferritin such as Mori et al (1975), Neitsu et al(1979), and Jones et al(1980). Each researcher group had used different antiferritins for study differrent groups of cancers. Mori et al, used anti-HeLa cell ferritin. They obtained the serum samples from various types of cancers, ie, cancers of stomach, liver, rectum, lung, and others, the positive reaction of their study is 36.8 % (21 out of 57 cases). Neitsu et al, studied mainly the cancers of stomach and colon using anti-liver ferritin for the test. They reported high serum ferritin level in 8 out of 18 patients

studied (44.4 %). Other study by Jones et al, they used both anti-spleen and anti-HeLa cell ferritin for study. Their results showed that the serum ferritin level in cancers of stomach and esophagus compared with normal subjects with high overlapping rate when they used anti-spleen ferritin for the study. Also, their results using anti-HeLa cell ferritin was unsatisfactory because it yielded very low level of serum ferritin from both cancers groups.

From these all evidences and our limited knowledge about the nature of ferritin in various cancers. It may be concluded that the possibility of serum ferritin for determination of general kinds of cancers were still not appreciated.

From the previous study, a definite achievement was reached by using ferritin from leukemic cell for the diagnosis of leukemia (27,30,31), and this ferritin was also useful for follow-up of leukemia patients during and after treatment (101). However, it was still doubtful in selecting the type of ferritin for diagnosis of solid cancer. But, the successful diagnosis in leukemia with ferritin will bring hope to researchers to find out the specific ferritin for solid cancer in the future.

The controversial idea for using the most suitable ferritin to be a tumour marker was still being discussed. The types of ferritins may be varied in various types of

cancers. It was not necessary that isoferritin must be changed to be a specific isoferritin for all cancers. For example, normal colon and kidney usually produce acid ferritin, but in cancer, this ferritin will be changed to be some types of basic isoferritin. In another hand, liver which normally produces basic isoferritin (40,42), but in cancer, this basic ferritin will be transformed to be some types of acid isoferritin(40,102). These isoferritins were altered because of the variation of subunit composition with different amino acid content (39,70,74). So, the antigenic determinance of ferritin was changed which in turn infleunce the test by immunological technique. However, the diversify of sialic acid composition has been reported to govern the isoelectric point value of each isoferritin (103).

According to the theory of "one gene one polypeptide" (104), it is possible that controlling gene of isoferritin was transformed to produce other isoferritin in cancer which it did not in normal stage. This is not the same as haematological conditions such as haemochromatosis whose gene was not altered but ferritin production was raised in order to convert free iron to be the storage protein iron.

The transformation of isoferritin in every kinds of cancers may not be neccessary to be the same isoferritin. In this same way, isoferritin of the same cancerous organ may not be similar. That is, one type of isoferritin may or may not be useful for diagnosis of all cancers. But the specificity of each isoferritin for each type of cancer may

be more possible. On the other hand, the cross reaction of each isoferritin type, using the combination of some suitably various antibodies against ferritins may be useful for screening and prognosis all kinds of cancers. Further studies should be undertaken with great efforts, continually.

Further Study

Ferritin may be an efficient and suitable tumour marker for a cancer or cancers if ones could find out a specific isoferritin for the test. The exact nature of cancer ferritin should be studied such as by the isoelectric focusing technique in both tissue and serum. These results could be compared between cancers and other diseases of the same organs. The differrence of isoferritin by the IEF studies between cancers and other diseases may reveal the specific isoferritin that can be used as a tumour marker for varieties of cancers. The use of this specific tumour marker is one of the best possible directions. And the hybridoma technique should be used for producing monoclonal antibody of the specifis isoferritin of cancer cells.