The Correlation Between Tnm and YY1 and P53 mrna Expression in nasopharyngeal Cancer

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The Correlation Between TNM and YY1 and P53 mRNA Expression in Nasopharyngeal Cancer

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Nasopharyngeal cancer is the fifth most severe malignant disease in the head and neck in the human body. The main treatment is chemoradiotherapy. The protein gene 53 (p53) is a tumor suppressor gene. YinYang1 (YY1) is a transcription factor having an important role in cell cycle control. YY1 can function as an activator, suppressor or initiator of the gene transcription process. This research aims to see the relationship between p53 mRNA gene expression and YY1 mRNA gene expression on the NPC TNM Stage. Materials and methods cross-sectional research at 20 WHO type 3 NPC in which 3 samples after chemoradiotherapy, 17 non-chemoradio therapy samples consisted of 8 stage two samples, 7 stage three samples and 2 stage four samples. With RT-PCR, YY1 mRNA gene expression and p53 mRNA gene expression were measured, then the T-test was independent of the average chemoradiotherapy group. It was concluded, at a higher NPC stage, the level of YY1 mRNA gene expression was relatively higher while p53 expression was lower. Post-chemoradiotherapy levels of p53 mRNA gene expression were higher and YY1 expression was lower than in the non-chemoradiotherapy group.

Keywords: Nasopharyngeal cancer TNM stage; p53; YY1.

Nasopharyngeal carcinoma (NPC) is a malignancy which accounts for about 2% of squamous cell carcinoma of the head and neck. The NPC case is the fifth most frequent case after cervical cancer, breast cancer, lymphoma and skin cancer. In this journal it was said that NPC was associated with the EBV virus 100%.[1-2]

WHO divides NPC into 3 types namely type 1 horn-coated flat cell carcinoma, type 2 and 3 are flat cell carcinoma without horny layers. In fact, the most often found is undifferentiated type III NPC according to WHO classification.[3-8]

The p53 gene is a tumor suppressor gene or tumor suppressor. Initially, p53 was thought to be an oncogene because it was found in excessive amounts or overexpression in malignant cells, the p53 gene was able to inhibit cell growth caused by oncogenes and could inhibit the tumorigenic potential of cells in animals. This proves that p53 is a tumor suppressor gene.[8-11]

Yin Yang1 (YY1) is a multifunctional protein that can act as a transcriptional repressor, activator, or initiator element binding to a protein that directs and initiates transcription in vitro.[12] YY1 is a ubiquitous & multifunctional zinc-finger transcription factor, which has an important role in the control of the cell cycle. YY1 has a regulatory role in cell growth, development and differentiation by influencing the levels of Cyclin D1, c-Myc, Rb, MDM2 and p53. YY1 is able to do negative regulation on p53 by increasing Murine Double Minute 2(MDM2)-p53 interaction which will lead to ubiquitination and degradation of p53. It is estimated that YY1 may regulate approximately 10% of the total genes in humans. In addition, Zaravinos who examined the expression of YY1 in tumors in humans explained that YY1 has a role as an inhibitory signal in p300 which is a co-activator of p53.[13-17]

The management of NPC varies based on the staging of the NPC itself. The staging system used for NPC was created by the American Joint Committeeon Cancer, based on the TNM system.[8]

This study was conducted with the aim of analyzing the relationship between YY1 mRNA gene expression and p53 mRNA gene expression in NPC patients to be assessed qualitatively using RT PCR analysis and its relationship to TNM staging. With this new exploration, it is hoped that it can be the basis for assessing the severity and further research in efforts to treat nasopharyngeal cancer.

METHOD

This research uses cross sectional study. The study was conducted on patients who came before or after the action of chemoradiotherapy at Siloam Hospital, Dharmais Hospital, PresidentialHospital atJakarta, Indonesia diagnosed with nasopharyngeal cancer and the Molecular Biology and Immunology Laboratory of the Faculty of Medicine, Hasanudin University, Makassar, Indonesia. Data collection will be conducted in the period of December 2018 to April 2020. The total number of subjects included in this study are 20 patients with diagnosed as nasopharyngeal cancer.

Using the quantitative real time PCR, samples was measured the levels of p53 mRNA gene expression and YY1 mRNA gene expression for each samplewith triplicate according to previous several studies.[18-23]

Statistic analysis

Statistical analysis was performed comparing the mean expression of p53 mRNA gene between groups of stages as well as the mean expression of mRNA YY1 genes between stages groups using independent T Test.[24]

RESULT

The youngest respondents were 27 years old and the oldest was 64 years with an average (mean) and median age of respondents 47 years. The number of samples is 12 men 8 women. Histopathology all subjects are WHO type III.

The sample studied consisted of 20 consisting of 17 who had never had chemoradiotherapy and 3 samples after chemoradiotherapy. Of the three samples, one had cycplatin 3 cycles and 6600 cGy radiotherapy, one cisplatin 2 cycles and 6600 cGy radiotherapy, one cisplatin 2 cycles and 4000 cGy radiotherapy. Three samples that had been able to undergo chemoradiotherapy and their tumors regressed to be minimal tumoron TNM stages.[8]

In table 1 showed that grouped eight stadium II samples, seven stadium III samples, two stadium IV samples and three minimal stage samples or first stage (post-chemoradiotherapy).

Measurement of YY1 mRNA gene expression at stage after chemoradiotherapy showed a mean value of 7.910 (6.389-8.817). Stage II shows the mean 10.921 (9.649-11.71). Stage III has an average of 11.816 (9.269-14.44) and Stage IV has an average of 13.976 (13.143 – 14.809) (table 2)

Measurement of the p53 gene showed a mean in stage post-chemoradiotherapy of 12.924 (11.421-13.975), Stage II had an average of 9.146 (8.105-10.492). Stage III has an average of 7.881 (5.166-10.256) and the last stage IV has an average of 6.736 (5.838-7.634) (table 3).

The first group (we call Group A) consists of respondents who have a post-chemoradiotherapy stage and the second group (we call Group B) is stage two, then the second stage compared to the stage three, the second stage compared with stage four, stage three compared to stage four.

The post-chemoradiotherapy stage was compared with the stage three. The post-chemoradiotherapy stage was compared with the stage four. The post-chemoradiotherapy stage was compared with the combined two, three and four stage groups that were not yet chemoradiotherapy.

Because group A and group B are not samples from the same population, we will do the unpaired T test at á = 0.05. By conducting the Fisher Variance test we can find out the type of the Independent Two-Sample T Test used whether using the assumption of variance between samples is the same or different.

YY1 mRNA gene expression post chemoradiotherapy compared with YY1 stage II, with stage III, with stage IV and with combined stage II and III and IV, stage II with stage IV statistically respectively each proven to be lower.

Whereas Stage II YY1 mRNA gene expression was compared with YY1 stage III mRNA gene expression, stage III with stage IV was not statistically proven to be lower.

Expression of p53 mRNA gene post chemoradiotherapy compared with p53 stage II, with stage III, with stage IV, and with combined stage II and III and IV, stage II with stage IV statistically respectively each proved to be higher.

Whereas Stage II p53 mRNA gene expression was compared with p53 stage III mRNA gene expression, stage III with stage IV was not statistically proven to be higher.

DISCUSSION

Based on general carcinogenesis, the process of change into cancer is caused by mutations in the gene controlling the cell cycle. The controlling genes are protooncogen, tumor suppressor gene, and repair genes.Generally patients come to health facilities after stadium 2 and above.

Treatment for NPC is chemoradiotherapy. Chemotherapy makes the cell situation in a state of rapid division which will facilitate the effects radiation. The radiation process will reduce the expression of the YY1 gene mRNA in nasopharyngeal tumors via p300 which will trigger an increase in p53, known as protooncogen.[9-11,16]Changes in the mean expression of the YY1 mRNA gene in stage two increased in stage three and more increased in stage four but the samples that had received chemoradiotherapy decreased are seen in table 2.But statistically the increased

Table 1. Distribution of Age, Sex, Chemo radiotherapy, and TNM stadium of Nasopharyngeal Cancer

Age (year)	Sex	Chemotherapy	Radiotherapy	TNM	Stage
28	Male	0	0	T4N2aM0	4
27	Female	0	0	T2N2bM0	3
57	Female	0	0	T2N2bM0	3
46	Female	0	0	T2N2M0	3
38	Male	0	0	T4N2aM0	4
37	Male	0	0	T2N2bM0	3
16	Male	0	0	T3N2aM0	3
55	Female	0	0	T3N2aM0	3
46	Male	0	0	T3N2AM0	3
47	Female	0	0	T2N1M0	2
53	Female	0	0	T2N1M0	2
57	Male	3 cisplatin	33	T0N0M0	1
59	Male	2 cisplatin	33	T0N0M0	1
52	Female	0	0	T2N1M0	2
64	Female	0	0	T2N1M0	2
46	Male	0	0	T2N1M0	2
42	Male	0	0	T2N1M0	2
53	Male	0	0	T2N1M0	2
46	Male	2 cisplatin	20	T1N0M0	1
37	Male	0	0	T2N1M0	2

expression of the YY1 mRNA gene was seen to be in a higher stage only evident from stage two compared to stage four. This is because the change between stage two to stage three and from stage three to stage four is not very noticeable. So it can be stated there is a change in the process of

Table 2. The Differences of YY1 mRNA gene expression in NPC stages

Stadium	n	Mean	Variance	Tcount	p-value
Stadium PC and II					
Stadium PC	3	7.91	1.756	2.747	0.013
Stadium II	8	10.939	0.529		
Stadium II and III					
Stadium II	8	10.939	0.529	1.212	0.123
Stadium III	7	11.813	3.585		
Stadium II and IV					
Stadium II	8	10.939	0,529	4,816	0.001
Stadium IV	2	13.976	1.388		
Stadium III and IV					
Stadium III	7	11.813	3.585	1.97	0.072
Stadium IV	2	13.976	1.388		
Stadium PC and III					
Stadium PC	3	7.91	1.756	3.726	0.005
Stadium III	7	11.813	3.585		
Stadium PC and IV					
Stadium PC	3	7.91	1.756	200	0.007
Stadium IV	2	13.976	1.388		
Stadium PC and non PC					
Stadium PC	3	7.91	1.756	-3.778	0.001
Stadium non PC	17	11.656	2.602		

Table 3. The Differences of p53 mRNA gene expression in NPC stages

No. Stadium	n	Mean	Variance	Tcount	p-value
Stadium PC and II					
Stadium PC	3	12.924	1.71	4.691	0.009
Stadium II	8	9.146	0.631		
Stadium II and III					
Stadium II	8	9.146	0.631	1.531	0.082
Stadium III	7	7.881	4.227		
Stadium II and IV					
Stadium II	8	9.146	0.631	3.511	0.004
Stadium IV	2	6.736	1.613		
Stadium III and IV					
Stadium III	7	7.881	4.227	0.965	0.203
Stadium IV	2	6.736	1.613		
Stadium PC and III					
Stadium PC	3	12.924	1.709	3.853	0.002
Stadium III	7	7.881	4,227		
Stadium PC and IV					
Stadium PC	3	12.924	1.709	5.235	0.007
Stadium IV	2	6.736	1.613		
Stadium PC and non PC					
Stadium PC	3	12.924	1.709	4.546	0
Stadium non PC	17	8.341	2.701		

increasing levels of YY1 mRNA gene expression in accordance with an increase in a higher stage. So there is an increased correlation between staging with high levels of YY1 mRNA gene expression.

Statistically T-test samples that received mean chemoradiotherapy YY1 mRNA gene expression levels were lower than each mean YY1 mRNA gene expression in stage two, or stage three or stage four, as well as post-chemoradiotherapy samples compared to the combined groups of stage two, three and four or a sample group that has not received chemoradiotherapy. So it can be stated that the expression of the YY1 mRNA gene will be seen to be decreased if chemoradiotherapy is performed.[15]

The change in mean expression of p53 mRNA gene in stage two decreases in stage three and more decreases in stage four but the samples that have received chemoradiotherapy increased expression. But statistically decreased expression of p53 mRNA genes is seen according to a higher stage and is only evident from the stadium two compared to stage four. This is because the change between stage two to stage three and from stage three to stage four is not very noticeable. So it can be stated that there is a process of changing the level of p53 mRNA gene expression in accordance with an increase in a higher stage. So there is an increased correlation between staging and the low expression of the p53 mRNA gene(table3)

Samples that received chemoradiotherapy mean p53 mRNA gene expression appeared statistically higher T Test compared to each p53 mRNA gene expression levels in stage two, or stage three or stage four, as well as post-chemoradiotherapy samples compared to the combined groups of stage two, three and four. So it can be stated that the expression of the p53 mRNA gene will be seen to be increased if the chemoradiotherapy is performed because it will trigger an increase in p53 function (table 3).

Protein 53 or p53 is a polypeptide expressed or coded by the p53 gene that plays a role in maintaining cell integrity or genome integrity through transcription and translation processes. The p53 gene is a tumor suppressor gene or tumor suppressor. In cells that have mutations, the cell will express p53 to try to repair damaged cells in accordance with the composition of the previous normal amino acids because of its protoonchogen nature (table3).

Chemotherapy and radiation will affect ATR which is a serine / threonine specific protein kinase that is involved in sensing DNA damage and activates a site of DNA damage assessment, which leads to the timing of cell cycle capture.[25-27]

The mean expression of the YY1 mRNA gene in stages two, three and four appeared to be more increased whereas the expression of the p53 mRNA gene was more decreased in NPC before this chemoradiotherapy showed the process of destruction of the polypeptide chain which in the advanced stage was more severe.[28-30] The mean YY1 mRNA gene expression in the stage before chemoradiotherapy was higher than the mean YY1 mRNA gene expression post-chemoradiotherapy reverse the mean expression of p53 mRNA gene before chemoradiotherapy was lower than that after chemoradiotherapy showed that the process of chemoradiotherapy went well to improve cells with increased expression of p53 mRNA genes, which in turn the proto oncogenic mRNA genes.[25]

The role of p53 in the cell cycle greatly contributes to preventing cancer growth. Ablation of YY1 results in the accumulation of the amount of p53, so conversely if an overexpression of YY1 stimulates the reduction and degradation of the p53 gene so that endogenous levels of p53 decrease. YY1 fights p53 with various mechanisms including increasing p53 ubiquination and degradation of p53, blocking p53 acetylation, weakening the stabilization of p14ARF, and inhibiting the mediating transcription of p53.[9-10,18]

YY1 is a nuclear protein expressed in all tissues. The name "Yin Yang" represents two opposing functions, namely as a TF to act as a repressor or activator. YY1 is a TF belonging to the GLI-Krüppel class of zinc finger protein. This has a fundamental role in embryogenesis, cell proliferation, and differentiation. YY1 exerts its biological effects through its ability to transactivate or suppress gene expression, depending on the gene that binds it. Recent studies have shown that YY1 indirectly activates or suppresses gene expression without DNA binding, through interactions with histone modifiers and chromatin remodeling proteins. Besides binding directly to p53, YY1 also binds to Arf and Mdm2 and increases ubiquitination

and p53 degradation, so YY1 is a negative regulator of p53.[12,18]

It has been explained that YY1 and p53 are inversely related. Statistically, the relationship between YY1 and p53 gene expression has a large correlation. An increase in YY1 was followed by a significant decrease in p53.[9-10,12,18]

CONCLUSION

High levels of YY1 mRNA gene expression are associated with high stages of nasopharyngeal cancer. The low level of expression of the p53 mRNA gene is associated with TNM stage of high nasopharyngeal cancer. After chemoradiotherapy, low expression of YY1 mRNA gene and high expression of p53 mRNA gene were found compared to before chemoradiotherapy.

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Ethical clearance

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Conflict of interest

Nil

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