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The correlation between TNM and YY1 and p53 mRNA expression in Nasopharyng Cancer

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Abstract:	Nasopharyngeal cancer (NPC) is a malignant disease in the head and neck and is the fifth cancer in the human body. Radiochemotherapy is the first choice for handling NPC. The protein 53(p53) gene is a tumor suppressor gene. YinYang1 (YY1) is a ubiquitous & multifunctional zinc-finger transcription factor, which has an important role in cell cycle control. YY1 can function as an activator, repressor or initiator of the gene transcriptional process. The purpose of this study was to determine the relationship between the expression of the p53 mRNA gene and the expression of the YY1 mRNA gene against NPC TNM Stage. Material and methods cross-sectional research on 20 WHO3 NPC samples in the form of 3 samples after radiochemotherapy, 17 samples of non-radiochemotherapy is consist of 8 stage two TNM samples, 7 stage three TNM samples and 2 stage four TNM samples. With RT-PCR, YY1 mRNA gene expression and p53 mRNA gene expression were measured, then the independent T-test of the mean sample group radiochemotherapy. YY1 mRNA gene expression in NPC post-radiochemotherapy has a mean value of 7.910 compared to that which has never been radiochemotherapy with an average of 11.656. The results of this statistical T test have a p-value of 0.001 <0.05. NPC Stadium that descended after radiochemotherapy found expression of the YY1 mRNA gene also dropped. Expression of the p53 mRNA gene in NPC after radiochemotherapy had an average value of 12.924 compared with never before radiochemotherapy 8.341 The results of this statistical T test had a p-value of 0.000 <0.05. TNM Stadium NPC that descended after radiochemotherapy was found to be higher expression of the p53 mRNA gene. It was concluded, at higher NPC TNM stages, the level of YY1 mRNA gene expression was relatively higher. NPC Post-radiochemotherapy there was a high level of expression of the p53 mRNA gene compared to the non-radiochemotherapy group.

Cover Letter for submission of a paper to

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14 July 2020

Dear Professor/ Doctor/ Mr/ Ms Editors

We wish to submit our manuscript entitled **"**The correlation between TNM and YY1 and p53 mRNA expression in Nasopharyng Cancer**"** for consideration to Enfermería Clínica and there has been no significant financial support for this work that could have influenced its outcome. We confirm that this work is original and has not been published elsewhere nor is it currently under consideration for publication elsewhere.

In this paper, we present data that higher NPC TNM stages, the level of YY1 mRNA gene expression was relatively higher. NPC Post-radiochemotherapy there was a high level of expression of the p53 mRNA gene compared to the non-radiochemotherapy group.. Please address all correspondence concerning this manuscript to our corresponding author at hattaram@yahoo.com.

Thank you for your consideration of this manuscript.

Best wishes,

Prof. Mochammad Hatta, MD, PhD

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Authors' contributions

PRB, MH, and SPR initiated and designed the study. PRB, EK, MH and AB drafted the manuscript. PRB, MH, SPR, FXBS supervised the field activities and the microbiology work. PRB, ES, SPR, MH, and AB helped to collect isolates. All authors have read and approved the final manuscript.

The correlation between TNM and YY1 and p53 mRNA expression in Nasopharyng Cancer

ABSTRACT

Nasopharyngeal cancer (NPC) is a malignant disease in the head and neck and is the fifth cancer in the human body. Radiochemotherapy is the first choice for handling NPC. The protein 53(p53) gene is a tumor suppressor gene. YinYang1 (YY1) is a ubiquitous & multifunctional zinc-finger transcription factor, which has an important role in cell cycle control. YY1 can function as an activator, repressor or initiator of the gene transcriptional process.

The purpose of this study was to determine the relationship between the expression of the p53 mRNA gene and the expression of the YY1 mRNA gene against NPC TNM Stage.

Material and methods cross-sectional research on 20 WHO3 NPC samples in the form of 3 samples after radiochemotherapy, 17 samples of non-radiochemotherapy is consist of 8 stage two TNM samples, 7 stage three TNM samples and 2 stage four TNM samples. With RT-PCR, YY1 mRNA gene expression and p53 mRNA gene expression were measured, then the independent T-test of the mean sample group radiochemotherapy.

YY1 mRNA gene expression in NPC post-radiochemotherapy has a mean value of 7.910 compared to that which has never been radiochemotherapy with an average of 11.656. The results of this statistical T test have a p-value of 0.001 <0.05. NPC Stadium that descended after radiochemotherapy found expression of the YY1 mRNA gene also dropped.

Expression of the p53 mRNA gene in NPC after radiochemotherapy had an average value of 12.924 compared with never before radiochemotherapy 8.341 The results of this statistical T test had a p-value of 0.000 <0.05. TNM Stadium NPC that descended after radiochemotherapy was found to be higher expression of the p53 mRNA gene.

It was concluded, at higher NPC TNM stages, the level of YY1 mRNA gene expression was relatively higher. NPC Post-radiochemotherapy there was a high level of expression of the p53 mRNA gene compared to the non-radiochemotherapy group.

Keywords: Nasopharyngeal cancer TNM stage, p53, YY1

INTRODUCTION

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. NPC is usually caused by a combination of 3 effects of etiological factors, namely: 1.carcinogenic environmental factors 2.genetics 3.EBV. [3,4,5,6.,7,8]

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.[8,9,10,11]

Yin Yang 1 (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.[13,14,15,16,17]

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 Committee on 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.

METHODS

This research uses cross sectional study. The study was conducted on patients who came before or after the action of radiochemotherapy at Siloam Hospital, Dharmais Hospital, Presidential Hospital at Indonesia diagnosed with nasopharyngeal cancer and the Biomolecular Laboratory of the Faculty of Medicine, Hasanudin University, Makassar. Data collection will be conducted in the period of December 2018 to April 2020. The number of samples is 20.

With Real time PCR we measured levels of p53 mRNA gene expression and YY1 mRNA gene expression levels for each sample according to Hatta et al. [18,19,20,21]

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 samples are WHO 3. Table 1,

The sample studied consisted of 20 consisting of 17 who had never had radiochemotherapy and 3 samples after radiochemotherapy. Of the three samples, one had cycplatin 3 cycles and 6600cGy radiotherapy, one cisplatin 2 cycles and 6600cGy radioeraphy, one cisplatin 2 cycles and 4000 cG radiotherapy. In all three samples, YY1 mRNA gene expression was lower than p53 mRNA gene expression. This shows that YY1 post-radiochemotherapy is lower than p53 expression.

While 15 other samples that did not get radiation were found YY1 mRNA gene expression is higher than p53 mRNA gene expression. Table 1

Two of the 17 samples obtained lower YY1 expression compared to p53 before radiochemotherapy. Three samples that had been able to undergo radiochemotherapy and their tumors regressed to be minimal T. Table1

From the above data we grouped eight stadium II samples, seven stadium III samples, two stadium IV samples and three minimal stage samples or first stage (post-radiochemotherapy). Table 1

Measurement of YY1 mRNA gene expression at stage I (after radiochemotherapy) 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 I or post-radiochemotherapy 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

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.[22]

The first group (we call Group A) consists of respondents who have a post-radiochemotherapy stage and the second group (we call Group B) is stage two, then the second stage group (A) compared to the stage three group (B), the second stage group (A) compared with stage four group (B), stage three group (A) compared to stage four group (B). The post-radiochemotherapy (A) stage group was compared with the stage three (B) group. The post-radiochemotherapy (A) stage group was compared with the stage four (B) group. The post-radiochemotherapy (A) stage group was compared with the combined two, three and four stage groups that were not yet radiochemotherapy.

Because group A and group B are not samples from the same population, we will do the unpaired T test at $\alpha = 0.05$. Using the Leven Test for Quality of

Variance on both mRNA YY1 gene expression variables and p53 mRNA gene expression variables, we can find that the variance of data between group A and group B on both variables is homogeneous, so the interpretation of p-values from the table below is guided by the variance values same.

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.

In table 2, YY1 mRNA gene expression post radiochemotherapy compared with YY1 stage II mRNA gene expressions, post radiochemotherapy stage with stage III, post radiochemotherapy stage with stage IV, post radiochemotherapy stage 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.

In table 3, Expression of p53 mRNA gene post radiochemotherapy compared with expression of p53 mRNA gene stage II, post radiochemotherapy stage with stage III, post radiochemotherapy stage with stage IV, post radiochemotherapy stage 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 YY1 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. Various types of proteins expressed in NPC are associated with the EBV virus.

Treatment of NPC is based on the tumor stage. Generally patients come to health facilities after stadium 2 and above. Stage one is rarely found to be treated. Treatment for NPC is radiochemotherapy. Chemotherapy is carried out in conjunction with radiotherapy to strengthen the function of radiotherapy. Chemotherapy makes the cell situation in a state of rapid division which will facilitate the effects of 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.[9,10,11,16]

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 radiochemotherapy decreased are seen in table 2.

But statistically the increased 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 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 due to YY1 gene as an oncogene.

Statistically T-test samples that received mean radiochemotherapy 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-radiochemotherapy samples compared to the combined groups of stage two, three and four or a sample group that has not received radiochemotherapy. So it can be stated that the expression of the YY1 mRNA gene will be seen to be decreased if radiochemotherapy 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 radiochemotherapy 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 due to the p53 gene as a protooncogen.

Samples that received radiochemotherapy 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-radiochemotherapy 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 radiochemotherapy 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.

The high expression of the YY1 mRNA gene at an advanced stage will suppress the expression of the p53 mRNA gene. E2F1 is an activator gene that encodes a family of transcription factors (TF) in higher eukaryotes.

P14ARF alternative reading frame called tumor suppressor ARF, ARF, p14ARF is a protein product of an alternative reading framework of the CDKN2A locus (eg the INK4a / ARF locus). p14ARF is induced in response to increased mitogenic stimulation, such as growth signals that deviate from MYC and Ras (protein).[4]

Mouse double minute 2 homologous (MDM2) also known as E3 ubiquitin-protein ligase. Here there is a change in function whether suppressor or stimulator of nasopharyngeal tumor oncogene from YY1 that affects p53 degradation.

E2F gene consists of 9 types 3 types as triggers oncogenes and 6 types as suppression of oncogens in mammals.

Yin Yang 1 (YY1) is a transcription factor that has a dual function as a tumor suppressor and tumor promoter. One of its functions is to regulate transcription activation and is related to various cellular processes including cell differentiation, DNA repair, autophagi, cell survival, apoptosis and cell division. In 2 independent studies conducted on the effects of YY1 on ovarian carcinoma and cervical carcinoma had very contradictory results.

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.

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 radiochemotherapy showed the process of destruction of the polypeptide chain which in the advanced stage was more severe. The mean YY1 mRNA gene expression in the stage before radiochemotherapy was higher than the mean YY1 mRNA gene expression post-radiochemotherapy reverse the mean expression of p53 mRNA gene before radiochemotherapy was lower than that after radiochemotherapy showed that the process of radiochemotherapy went well to improve cells with increased expression of p53 mRNA genes, which in turn were proto oncogenic mRNA genes. .

Bauknecht et al studied and found that bonding with YY1 can suppress the main and reinforcing activities of induction by 12-O-tetradecanoyl-phorbol-13-acetate (TPA).[13] YY1 primarily fights the development of viruses that cause latent and persistent infections, including adenoviruses, Epstein-Barr virus, herpes simplex type 1, cytomegalovirus, and HPV. In most cases of viral infections, YY1

plays a role in maintaining the expression of viral genes at low levels. But if certain triggers or stages in the life cycle of a virus not affected by YY1 occur (usually mediated by other transcription factors), the YY1 effect can be ignored. For example, serum-responsive factor (SRF) which can release YY1 from its receptors, so it actually activates the expression of the virus. YY1 acts as an inhibitor of cell repair in NPC.

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.

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.

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

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 radiochemotherapy, low expression of YY1 mRNA gene and high expression of p53 mRNA gene were found compared to before radiochemotherapy.

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CONFLICT OF INTEREST

All authors declare no conflict of interest.

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Tabla 1

Table 1 Distribution of Age, Sex, Radiochemotherapy and TNM Stadium

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
44	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
37	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

Table 2

Table 2 The Difference in YY1 mRNA gene expression between NPC stages

No	Variable	n	Mean	Variance	T count	p-value	Results
1	Stadium PC and II						
	Stadium PC	3	7.910	1.756	-2.747	0.013	reject H0
	Stadium II	8	10.939	0.529			
2	Stadium II and III						
	Stadium II	8	10.939	0.529	-1.212	0.123	accept H0
	Stadium III	7	11.813	3.585			
3	Stadium II and IV						
	Stadium II	8	10.939	0.529	-4.816	0.001	reject H0
	Stadium IV	2	13.976	1.388			
4	Stadium III and IV						
	Stadium III	7	11.813	3.585	-1.970	0.072	accept H0
	Stadium IV	2	13.976	1.388			
5	Stadium PC and III						
	Stadium PC	3	7.910	1.756	-3.726	0.005	reject H0
	Stadium III	7	11.813	3.585			
6	Stadium PC and IV						
	Stadium PC	3	7.910	1.756	-5.200	0.007	reject H0
	Stadium IV	2	13.976	1.388			
7	Stadium PC and non-PC						
	Stadium PC	3	7.910	1.756	-3.778	0.001	reject H0
	Stadium non-PC	17	11.656	2.602			

Table 3 The Differences in p53 mRNA gene expression between NPC stages

No	Variable	n	Mean	Variance	T count	p-value
1	Stadium PC and II					
	Stadium PC	3	12.924	1.710	4.691	0.009
	Stadium II	8	9.146	0.631		
2	Stadium II and III					
	Stadium II	8	9.146	0.631	1.531	0.082
	Stadium III	7	7.881	4.227		
3	Stadium II and IV					
	Stadium II	8	9.146	0.631	3.511	0.004
	Stadium IV	2	6.736	1.613		
4	Stadium III and IV					
	Stadium III	7	7.881	4.227	0.965	0.203
	Stadium IV	2	6.736	1.613		
5	Stadium PC and III					
	Stadium PC	3	12.924	1.709	3.853	0.002
	Stadium III	7	7.881	4.227		
6	Stadium PC and IV					
	Stadium PC	3	12.924	1.709	5.235	0.007
	Stadium IV	2	6.736	1.613		
7	Stadium PC and non-PC					
	Stadium PC	3	12.924	1.709	4.546	0.000
	Stadium non-PC	17	8.341	2.701		